THE EFFECTS OF TARGETED TRICLOPYR APPLICATION ON HABITAT QUALITY IN BOREAL SASKATCHEWAN TRANSMISSION RIGHTS-OF-WAY

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ABSTRACT

Vegetation management along transmission rights-of-way in remote northern forests across Canada is challenging. Mechanical removal of vegetation is often ineffective as many boreal species regenerate rapidly upon physical disturbance. Limited information on herbicide impacts in northern regions and on boreal vegetation makes communicating risks and benefits to local stakeholders and Indigenous communities difficult. Herbicides directly enter the ecosystem through deposition on vegetation and soils following application. Treated vegetation can be a vector of contamination to browsing herbivores, and herbicides can indirectly enter the soil ecosystem upon leaf abscission. Litter decomposition is critical to soil nutrient cycling and ultimately ecosystem health. The indirect effects of herbicides on habitat quality in boreal ecoregions remains poorly understood. Working in collaboration with SaskPower and the Lac La Ronge Indian Band, the influence of targeted applications of the herbicides, Garlon RTU and Garlon XRT (active ingredient triclopyr) were studied in northern Saskatchewan.

Triclopyr drift and dissipation in foliage were assessed following a targeted low-volume foliar (Garlon XRT) or basal bark (Garlon RTU) application. Greater drift concentrations localized at the stem base were observed with basal bark treatments. These effects may be exacerbated with high stem density, especially in conjunction with sandy soil prevalent in northern Saskatchewan which increases the potential of herbicide mobility and off-target effects. Concentrations in foliage were higher following low-volume foliar applications but dissipated to 50% of initial concentrations within a week (DT_{50} = 5.7 days and DT_{90} = 34.6 days). A hazard quotient risk assessment for moose (*Alces alces*) and snowshoe hare (*Lepus americanus*) indicates browsing on triclopyr treated foliage with the residues detected in this study are unlikely to result in acute toxicity (extrapolated from concentrations that caused 50% mortality in rats and rabbits, respectively); however, long-term browsing may cause adverse chronic effects (extrapolated from the concentration with no observable effects in a two-generation reproduction rat study with a safety factor of 100). Basal bark application is ideal when stem density is lower and toxic effects for herbivores is of concern, and low-volume foliar applications are best suited in areas with higher stem density when off-target herbicide deposition is less acceptable.

The indirect impacts of triclopyr on habitat quality were also examined through litter mass loss and quality (carbon:nitrogen ratios) as was the response of boreal invertebrates (*Folsomia*

candida and *Oppia nitens*) in microcosms and avoidance tests. Higher concentrations of nitrogen (lower carbon:nitrogen) were observed in field treated foliage resulting from triclopyr repression of natural leaf senescence processes. Litter breakdown rates were not significantly different within a year of treatment despite nitrogen profile differences between field treated and untreated samples. Triclopyr concentrations entering the ecosystem upon leaf abscission were below conservative avoidance endpoints for boreal invertebrates. The triclopyr concentrations that caused 50% of tested *F. candida* and *O. nitens* to avoid treated litter were above field application rates. At field application concentrations there were no differences in survival and reproduction rates of *F. candida*. Therefore, field application rates of triclopyr are not expected to impair ecosystem services and habitat quality based on the parameters evaluated in these studies. The results from these studies suggest the overall risk of targeted triclopyr use in northern Saskatchewan rights-of-way is low. These findings have improved our knowledge concerning triclopyr use in boreal ecosystems to support risk communication and informed integrative vegetation management decisions.

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2,4-D	2,4-dicholorophenoxyacetic acid
2,4,5-T	2,4,5-tricholorophenoxyacetic acid
ADI	Average daily intake
a.e.	Acid equivalent
a.i.	Active ingredient
C:N	Carbon:Nitrogen
DDT	Dichlorodiphenyltrichloroethane
DT_X	Disappearance time for X% of original residue concentration
EC_X	Effect concentration (X% reduction relative to control response)
HPLC-MS/MS	High performance liquid chromatography with tandem mass spectrometry
HQ	Hazard quotients
IAA	Indole-3-acetic acid
ICx	Inhibitory concentration (X% inhibition of response relative to control)
IS	Internal standard
IVM	Integrative vegetation management
KCDA	Keewatin Community Development Association
LC_X	Lethal concentration (X% reduction relative to control response)
LLOQ	Lowest level of quantification
MCPA	2-methyl-4-chlorophenoxyacetic acid
NOEL	No observable effect level
QC	Quality control
ROW(s)	Right(s)-of-way
SPE	solid-phase extraction
t_{bp}	Time of breaking point
TBEE	Triclopyr butoxyethyl ester
TCP	3,5,6-trichloropyridinol
TMP	3,5,6-trichloro-2-methoxypridine
triclopyr acid	3,5,6-trichloro-2-pyridinyloxyacetic acid
TRV	Toxicological reference values

1. PROJECT RATIONALE

The ecological impact of herbicides on northern transmission lines is especially significant for many northern communities that hunt and gather on and near rights-of-way (ROWs). Some northern community members are reliant on resources offered by the boreal forest for their livelihood and many Indigenous communities consider traditional foods to be of great cultural importance (Cidro et al. 2015). The forest clearing under transmission lines can improve access for hunting and gathering. However, the impact of herbicides in northern boreal regions has not been widely studied. SaskPower has proposed the use of selective herbicide applications using the active ingredient (a.i.) triclopyr, on target species along the northern I3P (formerly I1K) transmission ROW. Triclopyr and end-use products have been registered in Canada since 1991 for ground application in industry and woodland sites, and as such SaskPower has a legal license for use (Stewart 1991); however, local communities have not granted permission for herbicide use on traditional territory. Limited research on triclopyr use in northern environments has proven challenging for SaskPower when addressing specific community concerns in their engagement process. Triclopyr has been extensively studied in temperate regions, but much less is known about the ecological impacts at northern latitudes (Newton et al. 2008). The rate at which microbial breakdown and surface photolysis occurs could be drastically reduced with the colder temperatures and shorter light cycles associated with northern Saskatchewan winters (Bentson & Norris 1991; Johnson et al. 1995; Newton et al. 2008; Barnes & Seefeldt 2009). Moreover, the impact of triclopyr on target and non-target vegetation species in northern ecosystems has yet to be characterized. Studying the effects of triclopyr on local ecosystems is necessary before IVM practices using herbicides can responsibly and effectively be implemented on northern ROWs.

Triclopyr poses little threat to humans and wildlife at low concentrations and selective herbicide applications like basal bark or low-volume foliar treatments reduce impacts to non-target vegetation (Stewart 1991; EPA 1998; Nowak & Ballard 2005b). Although targeted herbicide applications like basal bark are associated with smaller areas of off-target effects, they can have higher herbicide deposition at the immediate site of application. Quantifying the deposition extent and amount from selective herbicide applications (i.e., basal bark and low-volume foliar) is critical in determining the associated non-target impacts. Herbicides can directly enter the soil ecosystem through application deposition or gain access after translocating into foliage and indirectly entering

the soil ecosystem upon leaf abscission. Studying the translocation of herbicides in native plants within their environments is important for predicting efficacy and fate of herbicides within the ecosystem. Previous work in the northern region of the Yukon Territory has demonstrated maximum application rates for Garlon XRT (a.i triclopyr) were below the reproduction effective concentration at 25% for common boreal soil invertebrates (*Folsomia candida, Oppia nitens*, and *Enchytraeus crypticus*) (Jimmo et al. 2018). However, uncertainty remains regarding the indirect impacts of herbicides on habitat quality (Clark et al. 2009). Herbicide induced changes to habitat quality may negatively influence leaf litter decomposers and therefore breakdown rates (CEW 2018). The studies in Chapters four and five will provide information to support utilities and local stakeholders in decisions related to herbicide use on northern transmission rights-of way.

Working in partnership with SaskPower and the Lac La Ronge Indian Band, this project was intended to respond to concerns of local Indigenous communities regarding the use of triclopyr in northern Saskatchewan ROWs on traditional territory. Two four-month internships with SaskPower were completed to provide insight into utility integrated vegetation management (IVM) practices and challenges of communicating technical information to the public. During the early stages of project development, open discussions with members of the Lac La Ronge Indian Band, the Lands and Resources Management Board, and the Northern Saskatchewan Environmental Quality Committee were conducted to understand the context of their concerns, provide information, and respond to questions. Local concerns were largely related to potential toxicity to browsing wildlife and were integrated into this study while also addressing less explored topics including herbicide drift and translocation following basal bark applications and indirect effects of triclopyr to habitat quality in boreal ecosystems. Enabling fair participation in environmental decision-making related to traditional territory by investing in community engagement is a critical aspect in acquiring social licenses and ultimately effective IVM on ROWs.

2. BACKGROUND AND LITERATURE REVIEW

2.1. INTEGRATED VEGETATION MANAGEMENT

2.1.1. *Purpose*

Northern transmission line rights-of-way (ROWs) are required to deliver reliable power to northern communities and industries. Tall trees and shrubs cause issues if they contact active lines, resulting in structural damage of transmissions lines, power outages, and fires from contact or flashover (Yahner & Hutnik 2004). The management of ROW vegetation is also required to allow for routine maintenance access. Management of vegetation on transmission lines solely through mechanical methods can promote suckering and heavy reseeding of undesired vegetation leading to quick and dense regrowth (Nickerson 1992; Frey et al. 2003; Ilisson & Chen 2009). Moreover, forests adjacent to cleared ROWs provide seeds and suckers that can quickly recolonize the area. Consequently, short maintenance cycles are necessary for adequate control that can be expensive due to costs associated with labour, fuel, and equipment (BASF 2005; Yahner 2006; Johnson 2008). Recurring disturbances from repetitive maintenance requirements can also negatively impact wildlife habitats and increase soil erosion (Nickerson 1992; Johnson 2008).

Integrated vegetation management (IVM) is the process of establishing stable low-growing plant communities capable of resisting the growth of problematic tall trees and shrubs (Niering 1987; Meilleur et al. 1997; Nowak & Ballard 2005a). Manipulating vegetation cover is the most effective and practical approach of inhibiting tree invasion (Dreyer & Niering 1986; Bramble et al. 1991; Berkowitz et al. 1995; Meilleur et al. 1997; Yahner & Hutnik 2004). Multiple IVM approaches, often used in combination, are required to be effective for the various situations present along ROWs like surface water, sensitive species, or public and private ROW use. Effective IVM can include several mechanical removal techniques, chemical control involving herbicides, and biological treatments where competitive compatible ROW species are planted on ROWs to delay tree reestablishment (Barnes & Seefeldt 2009; USEPA 2016). Mechanical removal on ROWs in North America was the sole method used for vegetation maintenance prior to the 1940s (Brown 1995). Herbicides were then introduced to reduce sprouting and suckering of hardwood species and have been used in conjunction with mechanical methods for decades in more temperate regions like Massachusetts (Nickerson 1992), New York (Nowak 1990), and Pennsylvania (Yahner & Hutnik 2004) (Brown 1995; Wagner et al. 2004). Much more recently

IVM has also been implemented in more northern regions like many Canadian provinces, and Alaska; however, there is much less research on the effects in boreal ecosystems (Wagner et al. 2004; Barnes & Seefeldt 2009). Biological treatments have been used to a much lesser extent than mechanical and chemical methods, but in Canada were proven effective in southern regions of Quebec (Meilleur et al. 1994) and Ontario (Brown 1995). Integrative vegetation management has the potential to be a more cost effective and sustainable approach to ROW maintenance that can minimize non-target effects and support long-term habitats for species (Nowak & Ballard 2005a; Yahner 2006).

2.1.2. *Chemical Control*

Integrative vegetation management that includes the use of herbicides can reduce the mechanical upkeep required and provide a reset of the local vegetation community, so that it can be transitioned into an alternative desired and stable state (Niering 1987; Pickett et al. 2009). Herbicides are lethal to the entire plant including the belowground portion and therefore are more effective at reducing stump and root sprouts than mechanical methods (Niering & Goodwin 1974; Bramble et al. 1991; Berkowitz et al. 1995; Ilisson & Chen 2009; Thiffault & Roy 2011). This is particularly important in boreal forests where many species like birch and aspen have adapted to fire disturbances and readily regenerate by sprouting and suckering after aboveground disturbances (Ilisson & Chen 2009). The choice of herbicide application technique depends on several factors including the plant species present, land accessibility and use, proximity to vulnerable areas like cropland or surface water, and the extent of vegetation control required.

There are several chemical control application techniques employed to manage incompatible vegetation species along ROWs including foliar spray, basal bark, and cut stump. Foliar spray can be applied as either a high or low-volume application. High-volume foliar spray or broadcast applications are generally nonselective herbicide treatments using a boom sprayer or a hand-held spray gun and hose connected to a pressurized spray tank and mounted to trucks or all-terrain vehicles. Nonselective herbicide applications are advantageous in areas with a high density of incompatible ROW vegetation or for initial herbicide treatments. Theoretically, successive treatments will be more targeted and less frequent as low-growing vegetation species establish. Broadcast herbicide applications are efficient and effective at removing incompatible ROW vegetation and do not require a large crew or much time to treat vast areas. However, depending

on the terrain, proximity to vulnerable areas, and adjacent land use, broadcast applications are not always appropriate to implement. High-volume herbicide applications are associated with a greater potential of herbicide drift, sites left exposed to invasive species, and adverse non-target effects to the ecosystem including reduced species diversity (Niering & Goodwin 1974; Nowak & Ballard 2005b). Nonselective herbicide applications are also, if not directly associated, often publicly perceived as being an increased risk of adverse effects to human and ecosystem health (Nowak & Ballard 2005b).

Selective herbicide applications specifically target and treat incompatible vegetation and are preferred when target species are less dense and non-target impacts of herbicide use are of concern. Moreover, it has been demonstrated that selective chemical vegetation management can promote the establishment of ROW compatible vegetation (Niering & Goodwin 1974; Dreyer & Niering 1986; Bramble et al. 1991; Meilleur et al. 1994; Yahner & Hutnik 2004). A low-volume foliar spray technique uses a pressurized backpack sprayer to manually target foliage of incompatible ROW vegetation species. Basal bark herbicide applications also use a low-volume backpack or a hand-held sprayer to apply herbicide to the lower 30 cm of bark on individual stems. In Saskatchewan, these techniques are generally only used on trees under approximately 2 m in height, as it would be hazardous for tall vegetation to fall following treatment and standing dead trees and shrubs are aesthetically undesirable. Targeted herbicide applications of woody species over approximately 2 m in height employ a technique called cut stump, in which tall vegetation is cut to ground level and herbicide is applied to the stump using a low-volume backpack sprayer. This technique can also be mechanized using the wet-blade method, where foliage is mechanically removed, and herbicide is applied directly to the cut surface. Wet-blading is a less targeted approach ideal for areas where high visibility is necessary or regrowth is too tall for a broadcast herbicide application. Selective herbicide treatments take longer and require a larger crew to remove incompatible ROW species but have the advantage of being used in tough terrain or in areas where herbicide drift and non-target impacts are less acceptable. Selective herbicide applications can reduce non-target impacts and, in some cases, herbicide usage by up to 50% (Nowak & Ballard 2005a; Power et al. 2013).

Selective herbicide applications using the active ingredient triclopyr have been identified for potential use on northern Saskatchewan ROWs. Previous studies have found that triclopyr presents

minimal risk to humans and wildlife at low concentrations and selective herbicide applications like basal bark or low-volume foliar treatments have reduced access to non-target vegetation (Stewart 1991; EPA 1998; Nowak & Ballard 2005b; Clark et al. 2009). Triclopyr has been extensively studied in temperate regions, but much less is known about the ecological impact in the boreal (Rombke et al. 2006b; Newton et al. 2008). Determining triclopyr input into the ecosystem from targeted application methods including drift concentrations and dissipation rates in treated boreal species is critical for the identification of potential adverse effects. Herbicide treated foliage could be a direct vector of toxicity for browsing wildlife or into the soil ecosystem upon abscission. Less is understood regarding potential indirect effects to habitat quality that could influence ecological services like the disruption of litter decomposition dynamics that regulate soil fertility. Characterizing the effects of triclopyr on local ecosystems is necessary before IVM can responsibly and effectively be implemented on northern Saskatchewan ROWs.

2.2. TRICLOPYR

2.2.1. *Garlon RTU and Garlon XRT*

Herbicide effectiveness hinges on the ability of the active ingredient to reach the target site in plants where it will elicit the desired biological response. The delivery of the active ingredient to the target site is dependent on the absorption, translocation, and metabolism of the herbicide (Kirkwood 1993). Many active ingredients are sparingly soluble in water to increase biological membrane permeability, which promotes access to the site of action in plants and decreases environmental mobility (Stephenson & Solomon 2007). Substances with low water solubilities require more liquid to be adequately dispersed for practical application. Moreover, delivering the active ingredient to the site of action in adequate concentrations often requires additional enabling substances. Adjuvants are surfactants, stickers, humectants, and anti-evaporating agents added to herbicide formulations to enhance biological activity, widen the conditions the herbicide can be utilized under, or improve spray application integrity (Stephenson & Solomon 2007; Tse-Seng et al. 2009). The predominant adjuvants used in herbicide formulations are nonionic surfactants, crop oil concentrates, and organosilicons (Tse-Seng et al. 2009). The mechanism in which they enhance herbicide action involves effects on application distribution, penetration through the cuticle or stomatal pores, and tissue absorption (Kirkwood 1993). Nonionic surfactants are amphiphiles that remove the wax layer on leaves to reduce surface tension. Crop oil is refined and purified paraffinic

oil that facilitates herbicide absorption and penetration through plant cuticles, reduces ester volatility, and delays herbicide crystallization on leaves (Tse-Seng et al. 2009). Organosilicons are amphiphiles that promote water adhesion and spreading of spray droplets by reducing water surface tension on leaves. Many herbicides use the same active ingredient but differ in the formulants and adjuvants to facilitate absorption. These inert ingredients in product formulations are rarely disclosed on product labels and are not always tested as comprehensively for toxic effects as the active ingredients (Song et al. 2012; Hwang et al. 2015).

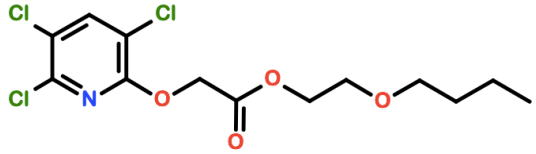
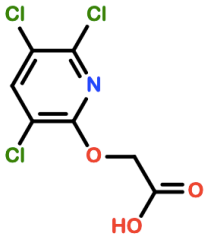
Garlon RTU and Garlon XRT manufactured by Dow AgroSciences both share the active ingredient (a.i.) triclopyr acid (3,5,6-trichloro-2-pyridinyloxyacetic acid). In both Garlon RTU and Garlon XRT, the active ingredient is formulated as a derivative of triclopyr acid, triclopyr butoxyethyl ester (TBEE), often referred to as the acid equivalent (a.e.). Triclopyr acid is reacted with alcohol to produce an oil-soluble ester, which is more lipophilic and capable of penetrating the lipoidal membrane of the plant cuticle or the bark of woody species through lenticels, pores, and natural bark breaks (Stephenson & Solomon 2007). Esters can be mixed with oil or water and emulsifiers to increase efficiency and improve delivery of spray application. Garlon RTU contains 200.3 a.i. g L^{-1} (144 g a.e. L^{-1}) and is packaged as ready to use with all added formulants and no further dilution required. Garlon RTU is a lipophilic formulation used as a basal bark or cut stump application for control of woody species and broadleaf weeds. Garlon XRT has 1050 a.i. g L^{-1} (755 a.e. g L^{-1}) and can also be used in basal bark or cut stump applications but is most often applied to foliage for control of woody species and broadleaf weeds. It is emulsifiable in water and is diluted before use to a concentration between 2.6 a.i. g L^{-1} for a single stem foliar application and 200 g a.i. L^{-1} for a basal bark application (Corteva agriscience 2020). Garlon XRT can be used alone or with additional adjuvants such as paraffinic oil and nonionic surfactants to improve spray consistency and control. Although the surfactant and concentration vary between Garlon RTU and Garlon XRT, the active ingredient is responsible for eliciting the desired biological response and is often the focus in most studies.

2.2.2. *Mechanism of Action*

The active ingredient in Garlon RTU and Garlon XRT, triclopyr, is a pyridine analog of the phenoxy herbicides. It elicits a response in plants by mimicking the endogenous auxin growth hormone, indole-3-acetic acid (IAA). Auxin and other auxinic herbicides like 2,4,5-

tricholorophenoxyacetic acid (2,4,5-T), 2,4-dicholorophenoxyacetic acid (2,4-D), 2-methyl-4-chlorophenoxyacetic acid (MCPA), clopyralid, picloram, dicamba, and chloramben induce diverse biochemical, physiological, and morphological responses in plants (Sterling & Hall 1997). The underlying mechanism of auxinic herbicides has not been characterized, but the response in plants is similar to that observed in plants with high concentrations of auxin in their tissues (Devine et al. 1993; Song 2014). Chlorophenoxyacetic herbicides are weak acids with low pKa values and readily dissociate in higher pH environments. The carboxylic acid on triclopyr has an approximate pKa of 2.68 (Table 2.1) and a greater proportion of triclopyr is dissociated into an anionic form as the environmental pH increases (McCall et al. 1988; Stewart 1991). It is speculated that triclopyr enters plant cells actively by auxin-binding proteins, as well as passively into the phloem due to its weak-acid properties (Hall et al. 1999). Auxin and auxinic herbicides then bind auxin receptors on the plasmalemma and induce a cascade of biochemical events within seven to ten minutes stimulated by the secondary messenger Ca^{2+}. The influx of Ca^{2+} into cells stimulates the plasma membrane H^{+} - ATPases triggering apoplast acidification and expansin activation (Devine et al. 1993; Cox et al. 2004). In acidic conditions, expansin is presumed to loosen the cell walls and promote turgor-driven cell enlargement, leading to plant growth by elongation (Cox et al. 2004). Within 30 to 40 minutes of auxinic herbicide exposure, nucleic acid and protein biosynthesis is also stimulated resulting in cell division and plant growth in some tissues (Cartwright 1976; Sterling & Hall 1997).

Table 2.1. Physical and chemical properties of triclopyr butoxyethyl ester and triclopyr acid.

Properties	**Triclopyr butoxyethyl ester**	**Triclopyr (acid)**
Molecular Formula	$C_{13}H_{16}Cl_3NO_4$	$C_7H_4Cl_3NO_3$
Structure*		
Molecular Weight (g mol^{-1})	356.7	256.5
Ionization Constant (pKa)	----	2.68† - 2.93‡
Melting Point (°C)	----	148 -150†‡
Vapor Pressure (mm Hg at 25 °C)	3.6 x 10^{-6} ‡	1.26 x 10^{-6}†‡
Henry's Law Constant (atm-m^3 mol^{-1} at 25 °C)	2.5 x 10^{-7}‡	9.7 x 10^{-10}‡
Octanol-Water Partition Coefficient at pH 7 (K_{ow})	12,300†	0.205† - 0.35§
Water Solubility (mg L^{-1} at 25 °C)	6.8‡ - 7.4§	430 - 440†‡
Soil Sorption Coefficient (K_{oc})	640 - 1780†§	25 - 134§
Half-life in Water (days)	0.5‡ - 2†	0.1 - 2§
Half-Life in Soil (days)	0.13 - 1.1‡§	10 - 56‡
Half-Life in Foliage (days)	1.1 - 15§	2 - 127|

Data obtained from the following sources:
* Royal Society of Chemistry (2020)
† Canadian registration decision for triclopyr via Agriculture Canada (Stewart 1991)
‡ United States registration decision for triclopyr via Environment Protection Agency (EPA 1988)
§ Triclopyr Human health and risk report for the USDA Forest Service (Durkin 2011)
| Bovey et al. (1986), Whisenant & McArthur (1989), Lewer & Owen (1990), Newton et al. (1990)

Excessive growth crushes phloem and cortical cells, causes xylem blockages, and stem epidermis ruptures that lead to diverse physiological responses (Sterling & Hall 1997; Hall et al. 1999). Those physiological responses observed in plants exposed to auxinic herbicides include cupping and stunting of leaves, cessation of terminal leaf growth, stem tissue proliferation, epinasty, stem bending and splitting, root thickening and stunting, and adventitious rooting (Devine et al. 1993; Sterling & Hall 1997). Triclopyr can also significantly increase the synthesis of the plant growth hormone ethylene, capable of inhibiting lignification and abscission in leaves and altering plant growth, resulting in physiological symptoms indistinguishable from auxin (Linck 1976; Kraus et al. 1991; Sterling & Hall 1997; Cox et al. 2004; Polko et al. 2011). Moreover, auxinic herbicides prompt metabolism events separate from IAA such as respiration inhibition through the chelation of heavy metal ions (Sterling & Hall 1997). Metabolic reserves are eventually exhausted leading to loss of cellular function, integrity, and repair capabilities and ultimately gradual plant death (Sterling & Hall 1997). Many studies have focused on the effects of triclopyr to crop species, but there is uncertainty on how those results translate to boreal species (Wagner et al. 2004; Rombke et al. 2006b; Newton et al. 2008; Clark et al. 2009). Sensitivities of boreal vegetation to herbicides are species dependent and often site-specific (Seefeldt et al. 2013). The biological response in plants to triclopyr is diverse and the absorption, translocation, and metabolism are dependent on vegetation species.

2.3. Fate in Vegetation

2.3.1. *Absorption*

The active ingredient, triclopyr, in Garlon RTU and XRT is formulated as the ester derivative, TBEE, to facilitate rapid absorption by vegetation (Lewer & Owen 1990; EPA 1998; Hall et al. 1999; Huang et al. 2000). Oil-soluble ester herbicides can increase the rate of herbicide uptake into waxy cuticles of leaves and can penetrate the bark of woody species through lenticels, pores, and natural bark breaks (Lewer & Owen 1990; Hall et al. 1999; Stephenson & Solomon 2007). Neutral lipophilic compounds like that of TBEE readily cross biomembranes. The addition of nonionic organosilicon surfactants to triclopyr formulations can enhance uptake. Foliar uptake of TBEE by wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*) was complete in 12 hours (Lewer & Owen 1990). Similarly, TBEE was rapidly absorbed by chickweed (*Stellaria media*) within the first 24 hours, followed by a slower sustained absorption that persisted for 72 hours (Lewer &

Owen 1990). Foliar absorption of triclopyr has been reported to fit the biphasic Michaelis-Menten model (Lewer & Owen 1990; Buick et al. 1992). It has been hypothesized that triclopyr acid is actively transported, contributing to the observed saturation kinetics. This is not an unreasonable assumption considering uptake of structurally similar 2,4-D has been speculated to occur on a carrier molecule like that which transports auxin. In contrast, the absorption of TBEE in water oak (*Quercus nigra*) and southern red oak (*Quercus falcata*) was found to be linear over 24 hours (Seiler et al. 1993). Enhanced triclopyr absorption is associated with foliage with little epicuticular wax, high stomatal and trichome densities, thin cuticle membranes, and when applied to the abaxial surface opposed to the adaxial surface of the leaf (King & Radosevich 1979). Following absorption, TBEE is quickly converted to the active herbicide form triclopyr acid (Lewer & Owen 1990; EPA 1998; Hall et al. 1999; Huang et al. 2000).

2.3.2. *Translocation*

Translocation of herbicides in plants involves the movement of the active ingredient from the site of uptake to another region in the plant through the xylem and phloem (Hall et al. 1999). The apoplastic pathway is composed of xylem vessels, non-living cellulose cell walls with a hollow lumen (Stephenson & Solomon 2007). The principal function of the xylem is to transport water and dissolved ions through intercellular space and porous cell walls from the roots to the shoots, where water is used by the leaves for photosynthesis or transpired. Water-soluble chemicals can be distributed into transpiring leaves or diffuse out of the xylem elements and into living cells. Toxicity is first observed where water loss is the greatest, at leaf margins. The phloem is the mass of continuous living vascular tissue interconnected by the plasmodesmata and enclosed by the cell membrane allowing for symplastic transport through cytoplasm (Hall et al. 1999; Stephenson & Solomon 2007). The phloem is specialized for the transport of photosynthesis products from leaves and primarily moves sugar from sites of photosynthesis to storage or active growth sites (Stephenson & Solomon 2007). The movement is usually downwards except during periods of rapid shoot growth. Herbicide is moved through the phloem with photosynthate and signs of toxicity are observed in the roots and in areas of rapid growth like shoot tips and younger leaves. In the spring, most sugar movement is upwards as woody plants pull up their root reserves for new growth. In the autumn, sugar and nutrients are pulled out of the leaves and moved down for storage in the stem and the roots. Therefore, autumn is usually the most effective season to apply herbicide

for hardwood tree control, as preventing root suckering and sprouting is dependent on translocation of the herbicide to the roots or rhizomes (Hall et al. 1999).

Translocation extent and distribution is highly influenced by plant physiology and environmental conditions (Song 2014). Triclopyr is primarily mobile in the phloem and accumulates in meristematic tissue (Radosevich & Bayer 1979; Stewart 1991; Hall et al. 1999). This was demonstrated in the examination of the translocation of ^{14}C-labeled triclopyr acid applied to both foliage and roots of big leaf maple (*Acer macrophyllum*), tanoak (*Lithocarpus densiflorus*), snowbush ceanothus (*Ceanothus velutinus),* bean (*Phaseolus vulgaris*), and barley plants (Radosevich & Bayer 1979). The ability of triclopyr as a weak acid (pKa 2.68) to ionize enables triclopyr to cross plasma membranes. Cytoplasmic pH in the phloem ranges between 7.5 and 8.0, surrounded by apoplast with a pH between 5.5 and 6.0. Triclopyr is predominantly protonated in the apoplast and capable of passing through the plasma membrane into the cytoplasm of the phloem where it becomes deprotonated in the less acidic solution. Dissociated acids have less of a tendency to diffuse through the plasmalemma and thus triclopyr is presumed to stay in the phloem and travel with the photoassimilates (Sterling & Hall 1997). However, depending on the plant species, triclopyr can translocate in the xylem for a time despite being in its protonated form before diffusing into the phloem. Three days after foliar application of ^{14}C-labeled triclopyr acid, the highest triclopyr acid concentrations detected in honey mesquite (*Prosopis glandulosa*) were primarily in the upper stem phloem then the basal stem phloem, the upper stem xylem, and the basal stem xylem (Bovey et al. 1986). Few studies have evaluated the potential of triclopyr to translocate into the roots. However, comparable concentrations of triclopyr acid were found in the roots and stems of honey mesquite (*P. juliflora*) 10 and 30 days after foliar application and triclopyr acid concentrations were found in the soil up to a year after stem injections to oak trees (Bovey & Mayeux 1980; Neary et al. 1988). Environmental conditions further exacerbate differences in translocation. Elevated temperature and photoperiods increase diurnal movement and water stressed woody plants have decreased translocation to stems and roots (Radosevich & Bayer 1979; Seiler et al. 1993). Studying herbicide translocation in ecologically relevant conditions is necessary as the extent and concentration of translocation is influenced by several factors including vegetation species type, the environment, and herbicide application technique.

Few studies have examined herbicide translocation in boreal species from basal bark application. Basal bark application of structurally similar 2,4-D was found to rapidly translocate from the treated area to foliage of approximately 1 m tall aspen trees (*Populus tremuloides*) within five days, although only a small percentage of the applied dose was observed (Eliasson 1973). This study showed a slow decrease in herbicide concentration in both dead and living tissue with 2,4-D residues remaining after two years of treatment. Further investigations are needed on the effects of triclopyr translocation from basal bark application in boreal species. The translocation into foliage may become a vector of contamination to browsing animals or disrupt litter decomposition dynamics upon leaf abscission that are critical to soil nutrient cycling. Studying the translocation of herbicides in native plants within their environments is critical for predicting not only herbicide efficacy, but also the fate within the ecosystem.

2.3.3. *Metabolism*

Once absorbed by vegetation, herbicidal phenoxyalkanoic esters like esters of 2,4,5-T and 2,4-D undergo rapid hydrolysis to phenoxyalkanoic acids (Smith 1976). The structural resemblance of 2,4,5-T to triclopyr acid suggests similar behaviour and has been demonstrated by the hydrolysis of TBEE to triclopyr acid within three days in wheat, barley, and chickweed following foliar application (Lewer & Owen 1987; Newton et al. 1990). Proton pumps on cell membranes transfer protons to the cell exterior, creating a more acidic environment outside the cell membrane (Hall et al. 1999). Weak acids tend to be protonated outside the cell wall, which increases herbicidal hydrophilicity and enables movement through the cell membrane. Once inside the cell, weak acids lose the proton and become hydrophilic and more water soluble, trapping the herbicide in the cell and making it available for phloem transport. Triclopyr acid (pKa of 2.68) predominantly exists in its anionic form under normal physiological conditions and can potentially accumulate in plant cells with relatively high pH like the phloem sieve tubes (Field & Dastgheib 1996; Foy 1996).

Following the hydrolysis of TBEE to triclopyr acid within vegetation, further breakdown is highly dependent on plant species metabolism. Despite the structural similarity of phenoxy herbicides to endogenous auxin, it is not metabolized in the same way. Moreover, phenoxy herbicides bind receptors with different affinities which influences plant sensitivity (Song 2014). There are only a few studies on triclopyr metabolism, but generally auxinic herbicides are

metabolized through oxidation, hydrolysis, and/or conjugation reactions (Sterling & Hall 1997). The carboxylic acid group on triclopyr acid binds to sugars like glucose, rhamnose, and galactose through an ester link and to amino acids like aspartate and glutamate through a peptide bond (Devine et al. 1993; Sterling & Hall 1997). However, triclopyr aspartate has been shown to hydrolyze back to triclopyr acid and consequently may not be a detoxifying mechanism (Lewer & Owen 1990). The metabolite 3,5,6-trichloropyridinol (TCP) has also been identified in the tissues of crop species with concentrations detected at least 10% below that of triclopyr acid (Lewer & Owen 1987; Norris et al. 1987; Brancato et al. 2017). The metabolism of triclopyr acid by cell suspension cultures of soybean (*Glycine max*) formed triclopyr aspartate and glutamate amide conjugates within seven days (Lewer & Owen 1987). The metabolism continued up to 21 days following the treatment and metabolites were predominantly retained within the cells. Within a day, 50% of the triclopyr acid concentration was metabolized in wheat and barley to a mixture of complex polar saccharide ester products, with the exception of 1-P-D-glucopyranoside (Lewer & Owen 1990). In the same study, metabolism of triclopyr acid in chickweed was slower, with a half-life of two days and formed relatively non-polar products like triclopyr aspartate. In a study with 2,4-D, metabolism in more tolerant species like grasses occurred through hydroxylation reactions that created irreversible detoxification products, whereas there was more of a tendency for reversible conjugations to amino acids in susceptible species (Barnes & Seefeldt 2009). Differences in metabolism processes between vegetations species results in variability in triclopyr acid responses including dissipation rates.

Triclopyr acid dissipation rates are relatively rapid in most species; however, there are reports of residues persisting for long periods. The focus of triclopyr acid dissipation has been predominantly in crop species because of the early use of auxinic herbicides in agriculture and in more temperate climates (Rombke et al. 2006b; Rüegg et al. 2007; Newton et al. 2008; Clark et al. 2009). Moreover, few studies examine the poorly characterized metabolites and products in vegetation. In Alaska, dissipation of triclopyr acid was relatively rapid in treated boreal species including salmonberry (*Rubus spectabilis*), blueberry (*Vaccinium ovalifolium*), high-bush cranberry (*Viburnum edule*), and red elderberry (*Sambucus racemosa*) with undetectable residues between 30 to 45 days (Newton et al. 2008). This dissipation is consistent with rates observed in several boreal species of more temperate climates with triclopyr dissipating to nearly half the initial concentration (DT_{50}) within six days (Whisenant & McArthur 1989; Thompson et al. 2000). In a

Yukon Territory study, the DT_{50} of a boreal willow (*Salix glauca*) was 11.5 days and declined to 16% of initial concentrations by 30 days after treatment (DAT) (Jimmo et al. 2018). Similarly, persistent concentrations have been observed in aspen foliage with 6 - 14% of initial concentrations remaining 120 DAT in Alaska (Newton et al. 2008). Concentrations persisted even longer in evergreen foliage with a DT_{50} of 127 days (Newton et al. 1990). It is notable that reported dissipation rates of triclopyr acid in plant species might also reflect losses due to rainfall wash-off, volatilization, and/or photodegradation in addition to plant metabolism (Cessna et al. 2002). Cooler temperatures and shorter photoperiods during a study may influence herbicide absorption and dissipation rates (Bentson & Norris 1991).

2.4. Vectors of Herbicide into the Environment

2.4.1. *From Vegetation to Soil*

A portion of any herbicide application eventually reaches the soil regardless of application method and can potentially impact non-target vegetation and soil fauna. Each herbicide application method has its advantages and disadvantages. Herbicide depositions from a low-volume foliar application average approximately 3.7 m from the site of application compared to the 7.3 m herbicide drift that can occur from a high-volume foliar application (Nowak & Ballard 2005b). Basal bark and cut stump applications have an average herbicide deposition of approximately 0.6 m from the site of application (Nowak & Ballard 2005b). Even conservative application methods that are associated with limited herbicide drift can exceed maximum application rates. Almost six times more herbicide is locally deposited from basal bark and cut stump applications than from high-volume foliar, which can result in 68 times more herbicide deposition when compared to low-volume foliar (Nowak & Ballard 2005b). In addition, basal bark and cut stump herbicide formulations often have higher active ingredient concentrations that can equate to the deposition of 100s to 1000s more grams of active ingredient compared with low and high-volume foliar applications, respectively (Nowak & Ballard 2005b). This can result in high residue deposition in the soil that could potentially adversely affect the soil ecosystem, especially in treatment areas with high stem density (Cessna et al. 2002; Holmes & Berry 2009).

Triclopyr butoxyethyl ester has a short residence time in all mediums and therefore the active ingredient of triclopyr and its intermediate breakdown products are often the focus of environmental studies. The hydrolysis of TBEE in soil to triclopyr acid occurs in approximately

one day (Table 2.1) (EPA 1988). The half-life of triclopyr acid is heavily influenced by soil properties and environmental conditions but has an average of 32 days (Table 2.1) (Barnes & Seefeldt 2009). The majority of studies have been performed in temperate conditions, but studies conducted under northern conditions have determined that triclopyr degradation is considerably reduced in winter months (Radosevich & Bayer 1979; Jotcham et al. 1989; Stephenson et al. 1990; Johnson et al. 1995; Thompson et al. 2000; Newton et al. 2008; Barnes & Seefeldt 2009). Studies in Alaska have detected triclopyr residues more than two years following application (Newton et al. 2008; Barnes & Seefeldt 2009). Triclopyr applied at the forest application rate in Yukon Territory soils persisted for more than a year, but dissipated to 50% of its initial concentration in one day and to 90% of its original concentration in three days (Jimmo et al. 2018). Although triclopyr residues can persist, concentrations are often below levels of human and ecological risk (Stephenson et al. 1990). Triclopyr acid is predominantly degraded to TCP in soil that has a half-life that can range between 8 and 279 days, again depending on soil properties and environmental conditions (Stewart 1991; Math et al. 2010; Maya et al. 2011; Cao et al. 2013; Li et al. 2013).

Herbicide dissipation can occur through a number of pathways including vegetation uptake and metabolism, volatilization, leaching, and runoff, but the primary mechanisms are through soil adsorption, microbial degradation, and photolysis (Stephenson et al. 1990; Johnson et al. 1995; Barnes & Seefeldt 2009). Triclopyr contains chromophores that absorb ultraviolet light within the solar spectrum (ie., wavelengths > 290 nm) and therefore is susceptible to direct photolysis in which bonds are cleaved or rearranged to form stable products (Eyheraguibel et al. 2009; Remucal 2014). Direct photolysis is a minor route of triclopyr dissipation on the surface of soil and the waxy films on leaves, although is a more predominant and rapid process of dissipation in water (Eyheraguibel et al. 2009). Indirect photolysis of herbicides can also occur where dissolved organic matter becomes a photosensitizer when irradiated and generates reactive species that accelerates degradation rates by creating a photo-Fenton reaction (McMartin et al. 2003; Remucal 2014). Indirect photolysis of triclopyr is not well characterized (Remucal 2014). Both processes are dependent on daylight hours and can be reduced in boreal regions in times of reduced daylight (Konstantinou et al. 2001; Graebing et al. 2003).

Degradation of triclopyr acid and intermediate products are mainly through microorganism breakdown (Stephenson et al. 1990; Johnson et al. 1995; Cessna et al. 2002; Berisford et al. 2006;

Newton et al. 2008). Microbial degradation rates of triclopyr are strongly influenced by soil temperature, moisture, and pH (Jourdan et al. 1998; Barnes & Seefeldt 2009). Lower temperatures reduce microbial activity ultimately leading to increased triclopyr and TCP residency time in soil (Johnson et al. 1995; Jourdan et al. 1998; Barnes & Seefeldt 2009). Increased soil moisture is positively correlated with microbial activity until a threshold limit is reached at which point the activity declines (Goetz et al. 1990; Johnson et al. 1995; Feng et al. 1998; Math et al. 2010). Moreover, soil-sorbed herbicides can degrade to a much lesser extent because of reduced availability to microbial degradation (Park et al. 2003). Herbicides can adsorb to soil through ionic, covalent and hydrogen bonding, Van der waals forces, ligand exchange, and hydrophilic bonding and partitioning (Gevao et al. 2000). Triclopyr (pKa 2.68) is deprotonated and negatively charged under most pH conditions and consequently, does not partake in much ionic binding with the negatively charged colloidal surfaces associated with organic matter and clay particles (Table 2.1) (Pusino et al. 1994; Johnson et al. 1995). However, the carboxylic acid groups of acidic herbicides such as 2,4-D, 2,4,5-T, and triclopyr become more protonated and can partake in additional hydrogen bonding with the hydroxyl and oxygen functional groups of soil organic matter at pH values below their pKa (Gevao et al. 2000). Acidic soil high in organic matter, often present in boreal forests, facilitate triclopyr sorption and decreases degradation rates, but generally triclopyr is only weakly sorbed to soil and therefore available for microbial degradation (Stougaard et al. 1990; Pusino et al. 1994; Johnson et al. 1995; Gevao et al. 2000). Microbial community structure and function can also be altered by soil acidity, most notably being that fungi thrive in low pH compared to bacteria and vice versa (Hogberg et al. 2007; DeForest et al. 2012). Optimal microbial activity usually occurs around neutral pH and any deviation can influence herbicide degradation rates (Jourdan et al. 1998). Herbicide bioavailability regulates degradation rates whether herbicide directly enters the soil ecosystem or gains access after translocating into foliage and indirectly enters upon leaf abscission.

2.4.1. *From Vegetation to Wildlife*

Herbicide treated foliage has the potential to be a vector of contamination for browsing wildlife. This is especially concerning in areas where hunting and gathering is prevalent near treated ROWs. Triclopyr acid and formulated TBEE are slowly absorbed through the dermis and are metabolized almost identically when ingested (Dost 2003). Triclopyr is primarily excreted by

the kidneys unchanged in the urine and therefore repeated exposure of sufficiently high concentrations can result in renal toxicity, but the risk of bioaccumulation is low (EPA 1988; Stewart 1991; Dost 2003). There is minimal risk of acute toxicity to wildlife through ingestion as the lethal concentration is generally higher than expected exposure rates (EPA 1988; Stewart 1991). In female rats, the lethal concentration that results in 50% mortality (LC_{50}) is 630 mg kg^{-1}. The triclopyr half-life was 3.6 hours and 89 - 95% was recovered in the urine following an oral dose of 60 mg a.i. kg^{-1} that saturated urinary excretion mechanisms (reversible renal effects) in rats (Timchalk et al. 1990). The triclopyr registry decision document by Agriculture Canada (Stewart 1991) recommends a conservative no observable effects level (NOEL) of 0.5 mg^{-1} kg^{-1} day^{-1} based on the most sensitive parameter measured in dogs that have an impaired ability to excrete acids (NOEL of 2.5 mg^{-1} kg^{-1} day^{-1} with reversible renal effects) (Timchalk et al. 1997). In comparison, no teratogenic or maternal toxicity is observed in monkeys, rabbits, or rats administered a dose of at least 25 mg^{-1} kg^{-1} day^{-1} and no chronic renal toxicity is observed in mice or rats between 3 - 5 mg^{-1} kg^{-1} day^{-1} based on a two year study (Stewart 1991). Agriculture Canada estimates that a rabbit may be exposed to triclopyr residues in vegetation of between 175 ppm and 813 ppm from typical applications that could equate to the consumption of 17.5 - 81.3 mg^{-1} kg^{-1} day^{-1} (Stewart 1991). Although prolonged exposure to these concentrations can cause sublethal effects, comprehensive reviews on herbicide toxicity suggest when considering herbicide degradation and dissipation kinetics, the risk of adverse effects to wildlife is unlikely at application rates (Stewart 1991; Lautenschlager & Sullivan 2002; Dost 2003; Clark et al. 2009). Moreover, wildlife is unlikely to exclusive browsing on herbicide treated ROWs, especially if suitable forage is available in the adjacent forest or ecotone as treated vegetation is less palatable (Joyal et al. 1984; Stewart 1991; Van Beest et al. 2010).

Conversely, the vegetation on ROWs can provide more desirable forage for browse animals and there are reports of increased browsing on ROWs compared to adjacent areas (Bramble & Byrnes 1982). Within the first year following mechanical removal and herbicide treatment, the area is first colonized by grass, herbaceous plants, and tree saplings. Shrubs, brush, and young trees then begin to take over and dominate for the next decade if left unmanaged. Moose (*Alces alces*) and white-tailed deer (*Odocoileus virgianus*) were observed browsing more intensively and black-tail deer (*Odocoileus hemionus*) deposited more pellets on treated ROWs compared to the adjacent forest (Bramble & Byrnes 1972; Loft & Menke 1984; Ricard & Doucet 1999; Gundula

et al. 2014). However, type and availability of surrounding foliage, width of ROW, and risk of predation can influence ROW use (Bartzke et al. 2014). In the first few years when vegetation cover is reduced there is a tradeoff between browsing on desirable ROW forage and predation risk. Increased browsing by wildlife on a ROW is typically observed when surrounding forage is less desirable or there is sufficient vegetation cover on the ROW to avoid predators (Bramble & Byrnes 1979; Loft & Menke 1984; Ricard & Doucet 1999; Gundula et al. 2014).

Prey can be susceptible in open corridors, especially in conditions of reduced vegetation cover like following vegetation management on ROWs (Joyal et al. 1984; Rieucau et al. 2009; Semeniuk et al. 2014; Andersen et al. 2017; Mumma et al. 2019). A study looking at risk perception of caribou (*Rangifer tarandus*) found a tendency to associate anthropogenic linear features with increased risk and thus an avoidance of these areas (Semeniuk et al. 2014). Similarly, an assessment of foraging costs on a ROW found that although white-tailed deer had trails that crossed the ROW perpendicularly, they were rarely active on the ROW outside the use of the trails during the winter months (Rieucau et al. 2009). In that study it was observed that although vegetation regeneration can increase food availability, the reduction in canopy cover increased snow depth making it more difficult to escape predators and resulted in reduced feeding on the ROW. Likewise, increased snow depth has been shown to restrict the movement and home range in moose and snowshoe hare (*Lepus americanus*) and be a deterrent of the use of areas with deeper snow (O'Farrell 1965; Peckarsky et al. 2008). White-tailed deer only browsed more on a ROW with higher stem density when that abundance exceeded six times the adjacent forest (Mayer & Carvell 1975; Bartzke et al. 2014). The ecotone between the ROW and adjacent forest can generate sufficient vegetation cover to shelter from predators and provide suitable forage (Bramble & Byrnes 1979; Joyal et al. 1984; Ferron & Ouellet 1992; Gundula et al. 2014). Moose and hare have been observed browsing more frequently on ROW ecotones than on ROWs (Joyal et al. 1984; Ferron & Ouellet 1992; Hodson et al. 2010; Hodson et al. 2011). Risk of herbicide toxicity to browsing wildlife is minimal considering the unlikelihood of exclusive browsing on herbicide treated ROWs, but it is difficult to sufficiently characterize this risk without realistic exposure predictions of relevant boreal vegetation. More studies that assess herbicide concentration in foliage following targeted herbicide applications in relevant boreal vegetation, as well as the complex browsing habits of various animals on anthropogenic linear features will improve risk assessments.

2.5. HABITAT QUALITY

Habitat is the environmental setting that directly supports the life, growth, and reproduction of a species, their population, and the entire community of species it contains (Southerland 1993). Variations in resources and environmental conditions influence the fitness of organisms and the ecological services they provide. Habitat quality is the ability of a habitat to support individual and population fitness (Hall et al. 1997). The fitness of soil and leaf litter dwelling organisms is interrelated with habitat quality, which is highly influenced by leaf litter and soil conditions. There are several elements and compounds in leaf litter that contribute to the growth and reproduction of the fauna; however, carbon, nitrogen, and phosphorous are most often used as indicators of litter quality (Krashevska et al. 2017). Nitrogen, carbon-to-nitrogen ratio, and lignin concentrations in leaf litter are most commonly used to characterize litter quality as they relate to rates of decomposition and nutrient release facilitated by decomposer organisms (Tian et al. 1992; Agren et al. 2013; Esperschutz et al. 2013; Krishna & Mohan 2017). Poor habitat quality can affect the sensitivity of organisms to environmental contaminants, further complicating the estimated risk associated with herbicide use. It is challenging to evaluate the contribution of soil and litter fauna to organic matter decomposition and nutrient cycling that regulate soil habitat quality (Rombke 2014). There are only two standardized tests that evaluate the activity of soil organisms in soil functioning and address the ecosystem services they provide (Rombke 2014). The bait-lamina *in situ* test is used to evaluate the habitat function of contaminated soils by measuring the distribution of macro and mesofauna and their related feeding activity, but with little emphasis on the contribution of microorganisms (Rombke 2014). The litterbag test measures mass loss of organic matter over time. This method allows the evaluation of the effect herbicides have on soil organic matter decomposition but does not differentiate between the contribution of invertebrates and microorganisms. The litterbag procedure provides a more obvious connection between feeding rate and soil function. Therefore, the litterbag test can be used to assess habitat quality as it relates to litter decomposition and decomposer organisms that facilitate nutrient release into the soil ecosystem.

The avoidance test examines the behavioural response of invertebrates to contaminants and is also reflective of habitat quality (Hund-Rinke et al. 2003; Hentati et al. 2013). This test is realistic to field conditions where invertebrates can use chemoreceptors to sense and potentially

avoid contaminants before adverse effects (Gainer et al. 2019a). Important ecosystem services may not be provided if a large enough invertebrate population avoids a contaminated area. The mechanism underlying soil invertebrate avoidance behavior is not well characterized and is complicated by contaminants partitioning into air, water, and/or solids (Hellou 2011). Nematode research indicates volatile compounds are sensed with olfactory cells while water-soluble compounds, such as salts and metals, are detected with gustatory cells (Gainer 2019). Avoidance responses are usually sensitive, rapid, and provide supplemental information regarding habitat quality to existing ecotoxicological tests (Loureiro et al. 2005b; Bori & Riva 2015). However, there have been reports suggesting this test is not always more sensitive than sublethal endpoints. For example, Collembola have displayed an attraction to contaminants with volatile odors like hydrocarbons (Bengtsson et al. 1988; Wenke et al. 2010; Gainer et al. 2019b). Avoidance tests can also produce variable responses like with gregarious Collembola where unexpected population shifts occur, but this can often be overcome with sufficient test replications (Bori & Riva 2015). Furthermore, chemosensing appears to be contaminant specific and vary between species. The soil avoidance test is a newer endpoint being used in soil ecotoxicology and has been standardized for springtails (*Folsomia candida)* and earthworms (*Eisenia fetida* and *Eisenia andrei)* (ISO 2008, 2011). The use of herbicide has the potential to directly (survival and reproduction) or indirectly (contaminant avoidance) compromise important ecological services provided by decomposer organisms like litter decomposition that contributes to nutrient cycling in the soil. Herbicide-induced changes to habitat quality can have long-term detrimental effects on ecosystem functions.

2.5.1. *Leaf Decomposition*

Detrital food webs regulate the decomposition of foliar litter and thus nutrient cycling in terrestrial ecosystems, including the transfer of fixed carbon to soil organic matter primarily facilitated by microorganisms (Gottschalk & Shure 1979; Bryant et al. 1998; Cambardella 2005; Kozlov & Zvereva 2015; Grubert et al. 2016). The nutrients made available to the soil system from litter decomposition accounts for up to 87% of nutrient requirements for forest plants (Krishna & Mohan 2017). The rate of leaf litter decomposition is initially rapid and driven by climate, nutrient concentration, and the species and abundance of decomposing organisms (Couteaux et al. 1995; Berg 2000; Gergocs & Hufnagel 2016). Within a few months, water-soluble compounds (7-30%) including sugars, phenolics, hydrocarbons, and glycerides leach out of the litter and are

sequestered by humus and clay (Couteaux et al. 1995; Berg 2000; Berg & McClaugherty 2003; Naiman et al. 2005; Gergocs & Hufnagel 2016). Microorganisms first transform easily degraded low-molecular weight compounds like non-lignified cellulose and hemicellulose (Couteaux et al. 1995; Berg 2000; Esperschutz et al. 2013). As decomposition progresses, carbon is respired and often exogenous nitrogen, a limiting resource in boreal ecosystems, is immobilized to meet microbial requirements and ultimately reduces litter carbon:nitrogen (C:N) over time (Aber & Melillo 1982; Parton et al. 2007; Berg 2014). Critical C:N ratios are generally between 20 and 40, above which nitrogen immobilization occurs and below which there is mineralization (Cabrera et al. 2005; Kim 2007; Liu & Sun 2013). In later stages of litter decomposition, rates are slow as recalcitrant substances such as cellulose and lignin are more difficult to mineralize and only few species can synthesize the ligninolytic enzymes involved (Melillo et al. 1982; Jana & Petr 2012; Esperschutz et al. 2013; Krishna & Mohan 2017). The quality or decomposability of litter is often determined using C:N and N:lignin ratios and is related to the activity of degrading microorganisms and soil health (Tian et al. 1992; Agren et al. 2013; Esperschutz et al. 2013; Krashevska et al. 2017; Krishna & Mohan 2017).

Herbicides can alter litter nutrient profiles that can impact decomposition rates and soil nutrient cycling dynamics. Auxinic herbicides inhibit natural leaf senescence and abscission processes including the translocation of nutrients into the roots and leaf lignification (Poovaiah 1974; Cromack & Monk 1975; Gottschalk & Shure 1979; Brown 1997). The outcome can be increased nitrogen (i.e., lower C:N) and lower lignin concentrations in herbicide treated compared to untreated foliage (Cromack & Monk 1975; Linck 1976; Gottschalk & Shure 1979). These conditions are favorable for microorganisms and high litter nitrogen concentrations have correlated with increased mass loss and earlier nitrogen mineralization (Bryant et al. 1998; Ehrenfeld et al. 2005; Naiman et al. 2005; Kim 2007; Laganiere et al. 2010). However, other studies suggest fundamental fungal activity may be repressed in litter with high nitrogen concentrations, inhibiting later stages of decomposition (Berg 2000; Zak et al. 2017). The foliage of white oak (*Quercus alba*) treated in midsummer with 2,4,5-T injection increased decomposition rates compared to controls as much as 50% resulting from herbicide-induced nutrient profile differences (Gottschalk & Shure 1979). Conversely, no difference in decomposition rates for spruce (*Picea glauca*) and red maple (*Acer rubrum*) foliage treated with 2,4-D and 2,4,5-T (100 ppm) were observed compared to untreated samples (Fletcher & Freedman 1986). Changes in litter chemistry can also

drive alterations in microbial community composition that can influence litter decomposition (Ferlian et al. 2015). If herbicides reduce key species or inhibit their functional capacity, leaf litter decomposition rates and essential ecosystem services will also be impaired (Schafer et al. 2012). Foliage decomposition rates can be influenced by several factors including environmental conditions, herbicide used, application timing, and the abundance of critical decomposer species making it difficult to predict outcomes.

Vegetation growth in a boreal ecosystem is often slow and limited by availability of nutrients and therefore the rate of nutrient release from leaf litter is key to productivity (Laganiere et al. 2010). Changing decomposition rates can cause an imbalance in the rate and timing of decomposition processes. Disruptions in litter dynamics like increased decomposition and nutrient release can lead to the reduction of the litter layer and can cause nutrient leaching and soil desiccation from thermal and moisture stresses (Gottschalk & Shure 1979). Nutrient depletion is especially problematic if there is a deficiency during important periods of plant growth and development. Conversely, retarded litter decomposition can cause undegraded organic matter to accumulate in soil (Berg 2000). Consequently, this could alter nutrient cycling dynamics and ultimately soil fertility that over time may select for new plant communities (Straková et al. 2012; Sollins & Gregg 2017). In either case, there are significant implications associated with altering decomposition dynamics using herbicides.

2.5.2. *Soil Invertebrates*

Soil invertebrates have a critical function in leaf litter breakdown and nutrient release. Mesofauna (0.1-2 mm) including springtails, mites, and enchytraeids are among the most abundant soil fauna and have the greatest direct impact on decomposition in forest ecosystems (Jensen 1974; Blair et al. 1992; Cambardella 2005). They inhabit air-filled pore spaces in soil and fragment and ingest plant litter, which improves leaf bioavailability and deposits nutrient rich fecal material into the soil (Carcamo et al. 2001; Yang et al. 2012; Buckingham et al. 2015; Gergocs & Hufnagel 2016). This creates a favorable environment for microorganisms and improves the soil water-holding capacity. In addition to mobilizing nutrients from organic matter, microbivorous soil invertebrates graze on bacteria and fungi influencing their quantity, composition, and activity (Sjursen et al. 2005; Lenoir et al. 2007; Krashevska et al. 2017). In more northern regions,

macrofauna are less abundant or even absent, making mesofauna key components to northern soil health (Sjursen et al. 2005; Aerts 2006).

Although many studies have focused on the ecological effects of herbicides like triclopyr, there have been few studies published specific to Canadian ecosystems examining the response of critical native microflora and fauna to herbicides (Braune et al. 1999; Gamberg et al. 2005; Rombke et al. 2006b; Princz et al. 2012). It is difficult to estimate boreal species sensitivities to herbicides with limited information on herbicide effects in northern Canada. Recent research has highlighted the importance of determining species native to the Canadian boreal and tundra regions that can be used in laboratory studies (Rombke et al. 2006b; Princz et al. 2012). The mesofauna profiles in northern boreal regions differ from other ecosystems, especially in that northern soil faunal generally have lower species diversity (Sjursen et al. 2005). Many ecotoxicity studies focus on the effects to earthworms, which are scarce or absent in northern climates (Sjursen et al. 2005; Aerts 2006; Loureiro et al. 2009). Collembola, Acari, and Enchytraeidae are all critical contributors to organic matter decomposition and are abundant in northern boreal ecosystems (Blair et al. 1992; Carcamo et al. 2001; Rombke 2003; Sjursen et al. 2005).

Boreal mesofauna are relatively insensitive to direct herbicide exposure. However, herbicide bioavailability and exposure is largely influenced by soil pH, organic matter content, and texture (Kuperman et al. 2006; Rombke et al. 2006a; Domene et al. 2011). In a study that focused on the effects of leaf litter treated with maximum application rates of 2,4,5,-T, the number of Collembola and Acari juveniles increased for up to eight months following application, but not enough to influence litter mass loss (Gottschalk & Shure 1979). Some observations report chlorophenoxyacetic acid herbicides can stimulate microbial populations and in turn stimulate microarthropod communities, but that is not always the situation as microbial abundance was unaffected in this study (Gottschalk & Shure 1979; Bolan & Baskaran 1996). Alternatively, sublethal affects from some herbicides have induced a hormetic response where there is an investment in survival of offspring through increased reproduction at the sacrifice of adult growth and individual survival (Van Straalen 1994; Puurtinen & Martikainen 1997). Toxicity testing with ecologically relevant boreal species including *Enchytraeus crypticus* (Enchytraeids), *Folsomia candida* (Collembola) and *Oppia nitens* (Oribatid mites) is critical for understanding the risk of herbicide use (Rombke et al. 2006b; Princz et al. 2010; Princz et al. 2012).

2.5.2.1. *Enchytraeus crypticus*

Enchytraeids, colloquially known as potworms, are small and unpigmented worms of the family Enchytraeidae (Rombke 2003; Coleman & Wall 2015). The species *Enchytraeus crypticus* (Oligochaeta, Haplotaxida, Enchytraeidae) is often used in ecotoxicological studies because of short generation cycles and high fecundity rates, and reproduction and survival soil toxicity tests have been standardized (Castro-Ferreira et al. 2012; Bicho et al. 2015). They have a high tolerance to a range of soil properties like pH, texture, and organic matter and can often be found on rich organic soils in the boreal forest and impacted sites (Rombke & Moser 2002; Rombke 2003; Coleman & Wall 2015). However, skin respiration makes them intolerant to low moisture soil conditions (Briones et al. 1997; Puurtinen & Martikainen 1997). They are involved in decomposition and humification processes in soil and feed primarily on decomposing plant material enriched with microorganisms, as well as the excrement of other soil fauna (Smith et al. 1990; Didden 1993; Rombke & Moser 2002; Rombke 2003; Coleman & Wall 2015). They inhabit the upper soil horizons in close association with soil pore water and can be exposed to contaminants through dermal contact, digestion, and respiration, making them great candidates for studying the impact of herbicides (Rombke 2003; Novais et al. 2010).

Enchytraeid toxicity from herbicides is strongly influenced by habitat moisture levels and herbicide water solubility. Enchytraeids cannot thrive with less than 10% moisture and increased toxic effects are observed in dry conditions (Briones et al. 1997; Puurtinen & Martikainen 1997). Additionally, their close association with soil pore water makes them particularly vulnerable to herbicides that are more water soluble (Puurtinen & Martikainen 1997). Adverse effects were only observed at high concentrations of the herbicides phenmedipham and atrazine that tend to have high soil sorption capacities (Novais et al. 2010). Similarly, the effects of the herbicides triclopyr and imazapyr on the lethal concentration that caused a 25% population decrease (LC_{25}) and concentration that reduced reproduction by 25% (EC_{25}) were above field application rates (Jimmo et al. 2018). Moreover, a hormetic effect with higher reproduction rates at low doses has also been reported (Puurtinen & Martikainen 1997; Arrate et al. 2002). Therefore, depending on habitat conditions and quality, *E. crypticus* may be adversely affected even at field application rates (Didden & Rombke 2001).

2.5.2.2. *Folsomia candida*

Collembola, or springtails, are among the most abundant terrestrial arthropod and commonly inhabit northern hemisphere boreal forests (Fountain & Hopkin 2005; Environment Canada 2014). The furca feature of the genus *Folsomia* enables them to jump from predators and has contributed to their common name of springtail (Fountain & Hopkin 2005). Reproduction, survival, and avoidance soil toxicity tests have been standardized for *Folsomia candida* (Collembola, Isotomidae) (ISO 2011; Environment Canada 2014). This parthenogenetic species is easily cultured in laboratory settings, short-lived with a lifespan of up to 140 days, and reproduces within 12-16 days after hatching with high reproduction rates (Environment Canada 2014). They are a major contributor to organic decomposition and soil respiration in boreal forests and consume a variety of food, but prefer fungal hyphae found on decaying leaf litter (Fountain & Hopkin 2005; Environment Canada 2007). They are also important prey species for soil predators like predatory mites, beetles, and centipedes (Fountain & Hopkin 2005). Similar to the Enchytraeids, *F. candida* are exposed to contaminants through dermal contact, digestion, and respiration and inhabit soil pore space in close association with soil pore water (Fountain & Hopkin 2005). The ease of handling this species in the laboratory combined with their global distribution and multiple potential exposure routes for contaminants make *F. candida* great candidates for herbicide toxicity tests.

The ventral tube feature of the *F. candida* facilitates fluid exchange and surface adhesion and makes them particularly sensitive to contaminants dissolved in soil pore water (Fountain & Hopkin 2005). Therefore, soils with high clay and organic matter contents can better buffer against toxic effects of herbicides like phenmedipham with a high soil sorption capacity (Fountain & Hopkin 2005; Diogo et al. 2007). Similarly, the herbicide trisulfuron had no adverse effects on survival or reproduction of another Collembolan species (*Onychiurus pseudogranulosus*) at field application rates and any toxicity observed above was determined to be from the herbicide formulation (Sabatini et al. 1998). Jimmo et al. 2018 also reported triclopyr and imazapyr LC_{25} and reproduction EC_{25} for *F. candida* to be above maximum herbicide application rates. However, herbicide properties and habitat conditions have been shown to influence *F. candida* sensitivities to herbicide exposure (Amorim et al. 2005; Domene et al. 2011).

2.5.2.3. *Oppia nitens*

Oribatid mites are in the largest family of mites, Oppiidae, and are the most abundant arthropods found in northern boreal forests (Crossley & Bohnsack 1960; Walter 1985; Blair et al. 1992). Oppiidae mites are critical to the ecosystem and *Oppia nitens* (Acari, Oribatida, Oppiidae) have recently been included in standardized survival and reproduction soil toxicity tests, but their long life cycles and low fecundity can make tests a challenge (Princz et al. 2010; Princz et al. 2012). Depending on temperature it can take between 21 and 46 days for a nymph to become an adult and their life span can extend beyond two years in cooler climates (Behan-Pelletier 1997; Princz et al. 2010). Oribatid mites inhabit the upper horizon of forest soils and are major contributors to organic matter decomposition, primarily feeding on decaying vegetation and fungi (Behan-Pelletier 1997; Princz et al. 2010). Moreover, their feeding habits and body structure assist in the dispersal of degrader microorganisms (Behan-Pelletier 1997). With few macrofauna present in boreal ecosystems, mites are critical to nutrient cycling and soil formation in northern soil which make *O. nitens* model candidates for herbicide toxicity tests (Behan 1978; Behan-Pelletier 1997).

Minimal toxicity tests have been conducted on the sensitivity of *O. nitens* to herbicides. Jimmo et al. (2018) found this boreal species to be tolerant to triclopyr with LC_{25} and reproduction EC_{25} well above the maximum field application rate. Unlike soft bodied Enchytraeids or Collembola, Oribatid mites have hard exoskeletons and dermal exposure to contaminants is hypothesized to occur through permeable joints in their legs (Gainer et al. 2018). To compensate for their lack of sight, they rely on physical cues and chemosensation to navigate through soil pores which may assist with an ability to sense and avoid contaminants (Orgiazzi et al. 2016). Adverse effects from contaminants can be exacerbated by soil conditions, especially organic matter which has a strong influence on reproduction rates and toxic stress of *O. nitens* (Maraun & Scheu 2000; Princz et al. 2010).

3. PROJECT OBJECTIVES AND HYPOTHESES

3.1. DRIFT FROM TARGETED HERBICIDE APPLICATION TECHNIQUES

The objective of this field study was to determine drift from targeted herbicide (triclopyr) application methods. I assessed this by quantifying the amount of triclopyr that drifts from the site of application due to a basal bark treatment of Garlon RTU and low-volume foliar treatment of Garlon XRT, applied via backpack sprayer. Hypotheses:

H_1: Triclopyr concentration due to a basal bark application of Garlon RTU would exceed the maximum allowable concentration for forest use, 4.53 kg a.i. ha^{-1}, at the base of the stem, but concentrations would be undetectable at 1 m from the site of application.

H_2: Triclopyr concentrations due to a foliar application of Garlon XRT would not exceed the maximum allowable label concentration, 4.53 kg a.i. ha^{-1}, but concentrations would be detectable up to 2 m from the site of application.

3.2. HERBICIDE CONCENTRATIONS IN ASPEN AND WILLOW LEAVES

The objective of this field study was to determine the concentration of herbicide (triclopyr) in foliage after targeted applications and assess if those concentrations would be high enough to cause adverse effects in browsing animals. Dissipation rates of triclopyr in leaves following basal bark application and low-volume foliar application were determined. I evaluated this by quantifying triclopyr and 3,5,6-trichloropyridinol (TCP) concentrations in leaves of *Populus tremuloides* saplings treated with a basal bark application of Garlon RTU for up to one year following application. I also quantified triclopyr and TCP residues in leaves of *Salix bebbiana* shrubs treated with a foliar application of Garlon XRT for up to one year following application. Hypotheses:

H_3: Triclopyr and TCP concentrations in leaves of *P. tremuloides* saplings treated with a basal bark application would be below the concentration that could cause adverse effects in browsing animals at all time points evaluated up to one year following application.

H_4: Triclopyr and TCP concentrations in leaves of *S. bebbiana* shrubs treated with a foliar application would be below the concentration that could cause adverse effects in browsing animals at all time points evaluated up to one year following application.

H_5: Triclopyr concentrations would be highest in *P. tremuloides* saplings and *S. bebbiana* leaves immediately following the foliar application and rapidly decrease by half the initial concentration within one week.

3.3. Effect of Herbicide Application on Litter Quality and Breakdown Rates

The objective of a field experiment was to investigate the influence of Garlon XRT (triclopyr) on breakdown rates and litter quality of *Salix bebbiana* leaf litter as determined by mass loss and carbon:nitrogen (C:N) ratios, respectively. Hypothesis:

H_6: Leaves of *Salix bebbiana* treated with a foliar application of Garlon XRT would have lower C:N ratios and faster breakdown rates over one year compared with untreated *S. bebbiana* leaves.

The objective of a laboratory experiment was to examine the influence of Garlon XRT (triclopyr) on leaf litter breakdown rates and quality of *Populus tremulodies, Betula papyrifera,* and *Salix bebbiana* leaf litter and evaluate the response of boreal decomposers (*Folsomia candida* and *Oppia nitens* reproduction and mortality) to treated litter. I achieved this through the use of microcosms by i) comparing mass loss and C:N ratio differences between herbicide treated and untreated leaf litter composites (*P. tremuloides*, *B. papyrifera*, and *S. bebbiana*) in litterbags and ii) comparing the mortality and reproduction differences of mesofauna (*F. candida*, *E. crypticus*, and *O. nitens*) residing in herbicide treated or untreated leaf litter. Hypotheses:

H_7: Foliage of *P. tremuloides*, *B. papyrifera*, and *S. bebbiana* treated with a foliar application of Garlon XRT would have lower C:N ratios and increased breakdown rates over four months compared with untreated litter.

H_8: Mortality rates of *F. candida*, *O. nitens*, and *E. crypticus* would not be significantly different in *P. tremuloides*, *B. papyrifera*, and *S. bebbiana* leaves treated with a foliar application of Garlon XRT compared to untreated litter; however, reproduction rates would be reduced.

The objective of a laboratory avoidance study was to determine if boreal forest mesofauna (*F. candida*, *O. nitens*, and *E. crypticus*) avoid leaf litter (*P. tremulodies, B. papyrifera,* and *S. bebbiana*) treated with Garlon XRT (triclopyr). I determined this using a 48-hour avoidance tests executed for each mesofauna species. Hypothesis:

H_9: The forest mesofauna, *F. candida, O. nitens*, and *E. crypticus*, would avoid treated *P. tremulodies, B. papyrifera,* and *S. bebbiana* litter by preferentially residing in untreated litter.

PREFACE: CHAPTER FOUR

Herbicide use in northern Saskatchewan has been limited and therefore challenges exist in communicating risks and benefits to local Indigenous communities and stakeholders. Targeted application of the herbicide triclopyr has been identified for use in boreal Saskatchewan rights-of-way and the first step in identifying risk is characterizing herbicide drift and dissipation. Triclopyr residues from drift and in boreal foliage following basal bark to aspen (*Populus tremuloides*) saplings and low-volume foliar treatments to willow (*Salix bebbiana*) shrubs, were examined under field conditions in boreal Saskatchewan. The triclopyr concentrations detected in boreal foliage were used in hazard quotient risk assessments for boreal wildlife of cultural importance, moose (*Alces alces*) and the snowshoe hare (*Lepus americanus*). The knowledge gained from these experiments will be used to support risk communication and integrative vegetation management decisions throughout northern Saskatchewan.

There is redundancy in the information provided in the background and literary review of Chapter One to provide context and support for the results of the experiments in this chapter.

Voinorosky C., Stewart K. Triclopyr Drift and Dissipation Following Targeted Herbicide Applications

Chelsea Voinorosky: Conceptualization, methodology, investigation, data curation, validation, analysis, writing (original draft), visualization
Katherine Stewart: Conceptualization, analysis, writing (review and editing), supervision, project administration, resources, funding acquisition

4. TRICLOPYR DRIFT AND DISSIPATION FOLLOWING TARGETED HERBICIDE APPLICATIONS

4.1. INTRODUCTION

Control of vegetation on northern transmission line rights-of-way (ROWs) is necessary in the delivery of safe and reliable electric service to northern communities and industries. Managing vegetation in boreal environments can be challenging, especially in rugged terrain and with an abundance of plant species capable of reproducing through suckering. Integrated vegetation management (IVM) is the adaptive approach of promoting desired, low-growing plant communities that are stable and resistant to the invasion of tall trees and shrubs (Niering 1987; Meilleur et al. 1997; Nowak & Ballard 2005a). An IVM plan that includes the use of herbicides can reduce the need for mechanical removal, which often leads to suckering and regrowth. Targeted herbicide application can also preserve off-target forbs and small woody shrubs, facilitating resistance to non-native grasses and tree invasion while also supporting early - mid successional growth stages that support a variety of wildlife species (Bramble & Byrnes 1979; Bramble & Byrnes 1982; Luken et al. 1991; Albert et al. 2013; Entsminger et al. 2019). Selective herbicide application in conjunction with mechanical methods has the potential to be a cost effective and sustainable approach to ROW maintenance that can minimize non-target effects and support long-term species habitat (Nowak & Ballard 2005a; Yahner 2006). Chemical methods of vegetation control have been widely used in temperate regions, but less so in northern latitudes (Niering & Goodwin 1974; Dreyer & Niering 1986; Bramble et al. 1991; Meilleur et al. 1994; Yahner & Hutnik 2004; Newton et al. 2008; Barnes & Seefeldt 2009; Douglass et al. 2016). Determining herbicide drift and dissipation from targeted application techniques in northern ecosystems is important in developing appropriate and effective IVM strategies.

Selective herbicide application requires more time and often more applicators to treat incompatible vegetation on ROWs; however, it has the advantage of being used in rugged terrain and in areas where herbicide drift and non-target impacts are less acceptable. Less drift and off-target impacts are associated with selective low-volume herbicide applications, but higher herbicide concentration can be localized around individual stems (Dreyer & Niering 1986; Nowak & Ballard 2005b). Basal bark treatments can be formulated with up to ten times more active ingredient in the formulated mix than foliar applied herbicides, contributing to 100s to 1000s of times more active ingredient per area from basal bark than from low or high-volume foliar

applications (Nowak & Ballard 2005b). One study reported 210 and 4198 times greater active ingredient at ground level within 0.3 m of basal bark applications than within 1.2 m of low and high-volume foliar applications, respectively (Nowak & Ballard 2005b). These effects can be exacerbated in areas with high stem density (Cessna et al. 2002; Holmes & Berry 2009). However, the most severe adverse non-target effects are often spatially limited (Dreyer & Niering 1986; Nowak & Ballard 2005b).

Selective herbicide applications using the active ingredient triclopyr acid, formulated as triclopyr butoxyethyl ester (TBEE), have been identified for potential use for northern ROW management. Triclopyr acid is often the focus of environmental studies because of the short residence time of TBEE in all mediums (EPA 1988). Triclopyr formulated as an oil-soluble ester (i.e. TBEE) is capable of penetrating the lipoidal membrane and facilities plant absorption, occurring between 12 - 72 hours in many crop species (Lewer & Owen 1990; EPA 1998; Hall et al. 1999; Huang et al. 2000). Once absorbed by vegetation, the hydrolysis of TBEE into triclopyr acid was reported within three days in wheat (*Triticum aestivum*), barley (*Hordeum vulgare*), and chickweed (*Stellaria media*) following foliar application (Lewer & Owen 1987; Newton et al. 1990). Further breakdown is highly dependent on plant species metabolism. There are only a few studies on triclopyr metabolism, but generally auxinic herbicides are metabolized through oxidation, hydrolysis, and/or conjugation reactions with sugar and amino acids (Devine et al. 1993; Sterling & Hall 1997). The common metabolites found in soil, 3,5,6-trichloropyridinol (TCP) and to a much lower extent 3,5,6-trichloro-2-methoxypridine (TMP), have also been identified in some vegetation tissue, although at a much lower frequency than triclopyr conjugations with sugars or amino acids (Lewer & Owen 1987; Norris et al. 1987).

Dissipation half-lives of triclopyr acid are generally relatively rapid, but there have been reports of residues that persist for long periods. These dissipation half-lives are often strongly influenced by vegetation species, but also by environmental conditions. The half-life of triclopyr acid in vegetation has been reported to range between 2 and 127 days (Bovey et al. 1986; Whisenant & McArthur 1989; Lewer & Owen 1990; Newton et al. 1990). Many studies have focused on the effects of triclopyr to southern crop species or in temperate regions, but there is uncertainty as to how those results translate to boreal species. Cool soil temperatures can slow down plant metabolism in winter months, potentially leading to increased herbicide persistence

compared to more temperate climates (Bonan 1989). Moreover, triclopyr breakdown occurs through volatilization, photolysis, and microbial means, all of which are reduced in colder temperatures and shorter light cycles associated with boreal winters (Bentson & Norris 1991; Johnson et al. 1995; Newton et al. 2008; Barnes & Seefeldt 2009). Herbicide sprayed on the leaf surface may dissipate more slowly in cooler climates before absorption, leading to higher absorbed herbicide concentrations. Herbicide treated leaves could be a vector of contamination to browsing wildlife and these risks also need to be characterized for boreal environments.

Triclopyr is predominantly excreted unchanged in the urine and therefore adverse renal effects are most common with high or persistent exposures. Sublethal effects to wildlife are more likely than acute toxicity as lethal concentrations are typically above what is observed in the field at application rates (lethal concentration that causes 50% mortality in rabbits is 550 mg kg^{-1}). The no observable effects level (NOEL) recommended by Agriculture Canada (Stewart 1991) of 0.5 mg^{-1} kg^{-1} day^{-1} was established from the most sensitive adverse renal effects observed in dogs that have a reduced capacity to excrete acids (NOEL of 2.5 mg^{-1} kg^{-1} day^{-1} with reversible renal effects) (Timchalk et al. 1997). Persistent exposure to treated vegetation has the potential to cause sublethal effects in wildlife (Stewart 1991).

Hazard quotients (HQ) can be used to measure the risk associated with wildlife browsing on triclopyr contaminated foliage. A HQ is the ratio of toxicity exposure to a toxicity reference value, such as a lethal concentration or a NOEL (with uncertainty factors applied to account for variables like inter-species extrapolations, if necessary) (Health Canada 2015). Hazard quotient risk assessment can be applied in a tiered approach, with each tier becoming less cautious and more realistic (Meek et al. 2011). In a tier-one assessment the toxicity exposure assumptions used are cautious and akin to a worse-case scenario, while in a tier-two assessment the exposure assumptions are more specific to the situation to advance more probable toxicity expectations (Meek et al. 2011). For example, a tier-two assessment may incorporate data that indicates the probability or frequency of an animal consuming contaminated foliage. Although ROWs can provide increased browsing, anthropogenic linear features can also leave prey vulnerable by facilitating predation efficiency (Joyal et al. 1984; Rieucau et al. 2009; Semeniuk et al. 2014; Andersen et al. 2017; Mumma et al. 2019). This is especially true within the first year following herbicide treatment when forage and cover is reduced. Boreal herbivores are unlikely to

exclusively browse on lower quality forage that follows the treatment of a ROW, especially if suitable forage is available in the adjacent forest or ecotone (Joyal et al. 1984; Ferron & Ouellet 1992; Hodson et al. 2010; Van Beest et al. 2010; Semeniuk et al. 2014).

Selective herbicide applications using the active ingredient triclopyr have been identified for potential use for northern ROWs management. Triclopyr has also been extensively studied in temperate regions, but much less is known about the ecological impact in northern latitudes and in boreal species (Newton et al. 2008). Characterizing the effects of triclopyr on local ecosystems is necessary before IVM can responsibly and effectively be implemented on northern ROWs. In this study, triclopyr residues from drift and in foliage following basal bark and low-volume foliar treatments to aspen (*Populus tremuloides*) and willow (*Salix bebbiana*), respectively were examined under field conditions in boreal Saskatchewan. The objective of this study was to quantify triclopyr drift and foliage concentrations after targeted applications and assess if those concentrations would be high enough to cause adverse effects in browsing animals. Determining drift and dissipation following targeted herbicide application will improve our understanding of the associated environmental risks in northern Saskatchewan, especially those risks associated with browsing wildlife.

4.2. METHODS

4.2.1. *Study Sites*

Drift and dissipation following basal bark treatment of aspen (*Populus tremuloides*) saplings and low-volume foliar treatment of willow (*Salix bebbiana*) shrubs were examined at two sites (Aspen and Willow, respectively). Both sites were located approximately 6 km apart on the PA8 transmission ROW along Highway 55 between Debden and Big River, Saskatchewan (Figure 4.1). The PA8 transmission line is one of the most northern Saskatchewan transmission lines where herbicide is currently applied, and it was selected to best emulate the vegetation and environmental conditions of more northern ROWs within Saskatchewan. Ecological site characteristics are provided in Appendix A.

Figure 4.1. Map of the Willow and Aspen field sites along the PA8 transmission ROW between the cities of Debden and Big River, SK, with red circled denoting sites and cities (QGIS Development Team 2020).

4.2.2. *Drift and Dissipation Study Design*

Herbicide drift was caught on watch glasses holding 9 cm diameter glass microfiber filter papers at four distances up to 0.7 m from twenty random aspen (*P. tremuloides*) saplings treated with a basal bark application of Garlon RTU (5.23 kilograms of active ingredient per hectare (kg a.i. ha^{-1})) on the Aspen site. The furthest distance of 0.7 m was selected to evaluate drift based on previous studies employing this application technique that determined deposition to be within 0.6 m of the application site (Nowak & Ballard 2005a) and overspray vegetation damage within 0.2 m to 0.4 m (Dreyer & Niering 1986; Nowak & Ballard 2005a). Herbicide drift was quantified at four distances up to 2.0 m from twenty random willow (*S. bebbiana*) shrubs treated with a low-volume foliar application of Garlon XRT (5.54 kg a.i. ha^{-1}) on the Willow site. The distance of 2.0 m was selected because previous studies using this application technique observed minimal

herbicide deposition (2.4 g ha^{-1}) between 1.2 m and 2.4 m from the application site (Nowak & Ballard 2005a) and overspray vegetation damage was within 1.0 m to 1.3 m (Dreyer & Niering 1986; Nowak & Ballard 2005a).

4.2.2.1. *Basal Bark Application – Aspen*

At the Aspen site, five plots delineated by natural clusters of *P. tremuloides* saplings were established (Figure 4.2). A study looking at clone size of 40-year old *P. tremuloides* in Riding Mountain National Park and Agassiz Forest Reserve, Manitoba, found an average clonal size of 28.5 m^2 and 7.79 m^2, respectively (Steneker 1973). A minimum distance of 32 m was considered sufficient to separate saplings of distinct genets and was the minimum distance at which the sampling plots were separated. There was a minimum of ten *P. tremuloides* saplings within each sampling plot (8-26 m in width) and all saplings within a plot were considered ramets of a genet. To assess drift, vegetation was cleared to ground level in a radius of 1 m surrounding the stems of twenty randomly selected *P. tremuloides* saplings. Vegetation was cleared to ground level prior to treatment to prevent non-target species from intercepting drift spray and therefore assuring maximum concentrations would reach the filter paper and provide a worst-case scenario. Watch glasses holding 9 cm diameter glass microfiber filter papers were placed immediately in front of and behind the stems at the point of treatment and at 0.3 and 0.7 m downwind prior to the basal bark application (Figure 4.3).

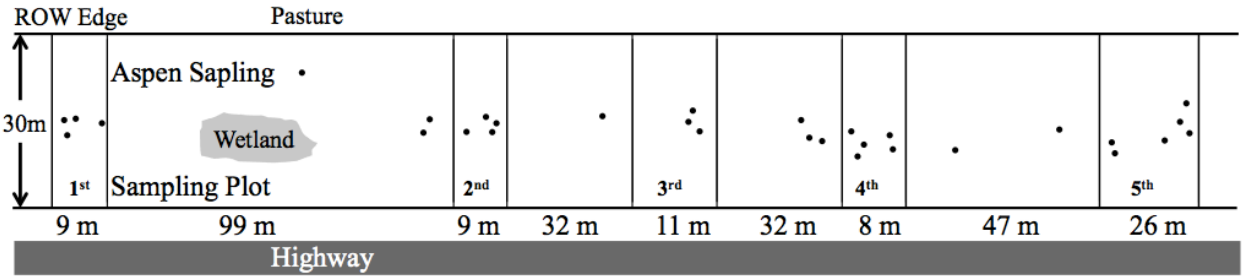


Figure 4.2. Study design for Basal Bark application of Garlon RTU (5.23 kg a.i. ha^{-1}) on the Aspen site located on the PA8 right-of-way between Debden and Big River, Saskatchewan. The leaves of aspen (*Populus tremuloides*) saplings (denoted by black dots) were destructively sampled from five plots (1^{st} - 5^{th}) at ten time points over the course of a year. Across the aspen site, twenty random *P. tremuloides* saplings were assessed for drift at four distances from the stem.

Figure 4.3. Drift of Garlon RTU applied by basal bark application on the Aspen site to twenty *Populus tremuloides* saplings with vegetation cleared to a radius of 1 m surrounding the stems. Watch glasses and filter paper were placed immediately in front of and behind the stems and at 0.3 and 0.7 m downwind prior to basal bark application.

4.2.2.2. *Aspen Dissipation Sampling*

Dissipation in treated *P. tremuloides* at the Aspen Site was assess by destructively sampling leaves from one to four saplings within each of the five plots at ten time points over a year. Individual *P. tremuloides* saplings were only harvested once to limit repeat sampling of a single clone. The leaves of untreated *P. tremuloides* individuals were also sampled at least 28 m from the treated ROW at every sampling interval to ensure no herbicide translocation over this distance was occurring. The majority of the *P. tremuloides* saplings that were treated and later sampled averaged 1.25 ± 0.43 m (n = 92) in height with a minimum height of 0.30 m and a maximum height of 3.00 m. Leaf samples (~30 g) were collected as soon as herbicides had dried on August 1, 2017 (~ 2 hours after application) and at 1, 2, 3, 7, 14, 29, 59, 91, 280, 366 days after treatment (DAT). Samples were collected in Ziploc bags, transported in coolers with icepacks, and stored at -20 °C until residue analysis. A subsample from the collected leaf material was weighed, oven-dried at 60 °C, and reweighed to determine dry mass.

4.2.2.3. *Low-Volume Foliar Application – Willow*

The Willow site was arranged around natural clusters of *S. bebbiana* shrubs on the PA8 transmission line along Highway 55 (Figure 4.4). A 90 m section containing at least twenty shrubs

was treated with a foliar application. A 60 m section with at least twenty shrubs was used as a control and left untreated. All shrubs to be sampled were at least 2 m apart and 3 m from the ROW edge. A 30 m untreated buffer zone where no sampling was conducted was established on either side of the untreated control area to ensure no herbicide drift or translocation occurred from the treated to the untreated area. The genet size of *S. bebbiana* and willow shrubs in general is poorly characterized. Typically, several meters will separate different genets of smaller willow species (Fritz & Price 1990; Douglas 1991). Due to the relatively close proximity of the willow shrubs across the entire site, samples were taken in composite across treated and untreated areas rather than attempting to define individual clones.

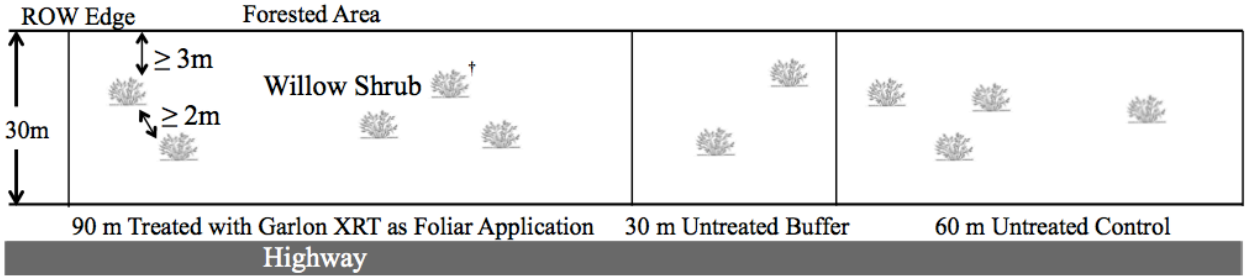


Figure 4.4. Study design for the Willow site located on PA8 ROW between Debden and Big River, Saskatchewan: Treated (90 m) with Garlon XRT (5.54 kg a.i. ha^{-1}) and untreated control (60 m) areas of the Willow site contained at least twenty willow (*Salix bebbiana)* shrubs and were separated by a 30 m buffer zone. †Pinehurst Floral & Greenhouse (2010)– Shrub Drawing (© 2020 PaintingValley.com), CC BY-NC 4.0 (Painting Valley 2020).

To assess drift at the Willow site, vegetation was cleared to ground level in a radius of 2 m surrounding the stems of twenty randomly selected *S. bebbiana* shrubs. Watch glasses holding 9 cm diameter glass microfiber filter papers were placed downwind immediately at the front of and at 0.5, 1.0, and 2.0 m from the stems prior to the foliar application (Figure 4.5). Immediately following both treatment methods, filter papers were placed in glass jars with acetone using tweezers, transported in coolers with icepacks, and stored at -20 °C until analysis.

Figure 4.5. Drift of Garlon XRT applied by foliar application on the Willow site to twenty *Salix bebbiana* shrubs with vegetation cleared to a radius of 2 m surrounding the stems. Watch glasses and filter papers were placed downwind immediately at the front of and at 0.5, 1.0, and 2.0 m from the stems prior to the foliar application.

4.2.2.4. *Willow Dissipation Sampling*

At the Willow site, five composite leaf samples from *S. bebbiana* shrubs were collected in the treated section at ten time points over a year. Each composite sample was composed of approximately equal amounts of leaves from four shrubs in immediate proximity. In addition, a composite sample of leaves from four shrubs in the untreated control area was collected at each time point to ensure no herbicide was detected. The *S. bebbiana* shrubs that were treated and later sampled averaged 1.59 ± 0.21 m (n = 20) in height with a canopy at the largest width of 1.56 ± 0.27 m and 32 ± 16 stems. The untreated control *S. bebbiana* shrubs that were sampled averaged 1.57 ± 0.28 m (n = 24) in height with a canopy at the largest width of 1.53 ± 0.41 m and 20 ± 13 stems. Willow leaf samples (~30 g) were collected as soon as the herbicide had dried on August 2, 2017 (~ 2 hours after application) and at 6, 13, 28, 58, 70, 90, 280, 315, 341, 365 DAT. Samples were collected in Ziploc bags, transported in coolers with icepacks, and stored at -20 °C until residue analysis. A subsample from the collected leaf material was weighed, oven-dried at 60 °C, and reweighed to determine dry mass.

4.2.3. *Herbicide Application*

Herbicide applications at the Aspen and Willow sites were applied by licensed applicators of Davey Tree Company of Canada Ltd., contracted by SaskPower. A low-volume and pressure (15-

85 psi) treatment using the SPS International Commercial YT-189 Backpack Sprayers with a flat-fan nozzle was used for both the basal bark and foliar applications. On August 1, 2017, the Aspen site was treated with a basal bark application of Garlon RTU (144 g L^{-1} triclopyr butoxyethyl ester; Dow AgroSciences Canada Inc, Calgary, AB) at a rate of 5.23 kg a.i. ha^{-1}. For basal bark application, herbicide was applied to the base of the *P. tremuloides* stems. Stems with a diameter less than approximately 5 cm were treated on one side of the stem, whereas stems with a diameter greater than approximately 5 cm were treated on both sides. At the time of application, the temperature was 20 °C and the wind velocity was between 5 km hr^{-1} and 7 km hr^{-1} to the South. On August 2, 2017, the Willow site was treated with a foliar application of Garlon XRT (755 g L^{-1} triclopyr butoxyethyl ester; Dow AgroSciences Canada Inc, Calgary, AB) at a rate of 5.54 kg a.i. ha^{-1}. At the time of application, the temperature was 14 °C and the wind velocity was 2 km hr^{-1} to the South.

4.2.4. *Herbicide Analysis*

Herbicide residue analysis of glass microfiber filter papers from the drift study, *P. tremuloides* leaves, and *S. bebbiana* leaves were conducted using modified procedures provided by Dow AgroSciences Canada Inc, Calgary, A.B. (Tessier 2013; Lynn & Slinkard 2014) and are described in detail in Appendix B.

Glass microfiber filter papers from the drift experiments were stored in acetone at -20 °C. The samples were evaporated with nitrogen to complete dryness using the TurboVap evaporator (Labconco; Kansas City, M.O.) set at 40 °C with nitrogen flow rate of approximately 15 psi. The filter papers from drift samples were acetone rinsed and samples were purified using a solid-phase extraction (SPE) procedure. Waters Oasis HLB 3cc (60 mg) extraction cartridges were used to purify Garlon XRT drift samples with a polymeric reversed-phase sorbent, rinsing with water and eluting with methanol. Phenomenex Strata-XL-A 100um (30 mg) extraction cartridges were used to purify Garlon RTU drift samples with a polymeric strong anion sorbent, rinsing first with 25 mM ammonium acetate, then with methanol, and eluting with methanol with 5% formic acid. Samples were again evaporated to complete dryness using the TurboVap evaporator and reconstituted in methonal:water (50:50, v/v) before analysis. The lowest level of quantification was 0.020 g ha^{-1} for Garlon XRT drift samples and 0.028 g ha^{-1} for Garlon RTU drift samples.

Leaves used for residue analysis were stored at -20 °C, freeze-dried, and homogenized using a ball-grinder (Retsch MM-400, Germany) before analysis. Residues of triclopyr and TCP were extracted from leaf matrices (0.25 or 0.50 g) by adding 5 or 10 mL of methanol/2.5 N sodium hydroxide (90:10, v/v) and shaking the sample for 60 minutes on a flat-bed shaker at approximately 180 revolutions per minute. A 0.2 mL sample extract was mixed with 0.8 mL methanol/2.5 N sodium hydroxide (90:10, vv) and 1.0 mL of 1.0 N hydrochloric acid by vortex. Phenomenex Strata-X 33um (30 mg) extraction cartridges were used to purify litter samples with a polymeric reversed-phase sorbent, rinsing acetonitrile/ultra-pure water/1N hydrochloric acid (30/69/1, v/v/v) solution and eluting with acetonitrile/ultra-pure water/1N hydrochloric acid (60/39/1, v/v/v) solution. The lowest level of quantification for triclopyr and TCP was 0.013 ug g leaf $^{-1}$.

All samples were analyzed using high performance liquid chromatography coupled with tandem mass spectrometry (HPLC-MS/MS) in the Health Sciences Department at the University of Saskatchewan. The accuracy and precision of the standard curves were assessed through replicate sets of low, medium, and high-quality controls (QC) spiked independently of calibration standards as described in Appendix B. Matrix extraction and dilution integrity were also assessed for triclopyr residues in leaf samples. The sensitivity of the methods was evaluated by comparing the area of the analyte or internal standard (IS) of a matrix blank to that of the lowest level of quantification (LLOQ) and were confirmed to be less than 20% (analyte) and 5% (IS). The LLOQ for drift samples was 6.0 ng mL^{-1} and the LLOQ for leaves was 2.5 ng mL^{-1}. All samples analyzed on the HPLC-MS/MS were spiked prior to purification by solid phase extraction with the stable isotope triclopyr [M+4] used as the internal standard. Every sample quantification HPLC-MS/MS analysis contained at least six calibration points with a correlation coefficient of $r \geq 0.98$. Each quantification sample was detected within 15% of nominal concentration, except for the LLOQ, which was within 20% as per guidelines (USFDA 2018). At least 67% of QCs (and 50% of QC at each concentration) were within 15% of the nominal value. Each HPLC-MS/MS analysis contained a pure solvent solution, matrix blank, matrix blank with internal standard, calibration standards (at least six), and QCs (making up at least 5% of the total unknown samples).

No TCP was detected in *S. bebbiana* or *P. tremuloides* leaves (LLOQ of 0.013 ug g leaf $^{-1}$). The full method for triclopyr and TCP validation and quantification are described in Appendix B.

4.2.5. *Statistical Analysis*

Triclopyr concentrations at different distances from the stem following a basal bark treatment (n = 19 at 0.7 m and stem front; n = 20 at 0.3 m and stem back) were analyzed using one-way analysis of variance (ANOVA) followed by a TukeyHSD post hoc test ($p<0.05$). Prior to ANOVA, herbicide concentrations were natural log transformed to meet assumptions of equal variance (Bartlett Test of Homogeneity of Variance, $p>0.05$) and normality was confirmed for the model residuals (Shapiro-Wilk Normality Test, $p>0.05$). Triclopyr concentrations at different distances from the stem following a low-volume foliar application method (n = 20 at 2, 1, 0.5 m and stem front) were analyzed using Kruskal-Wallis rank sum test followed by Dunn's post hoc test of multiple comparisons using rank sums ($p<0.05$). Drift samples that were non-detects (LLOQ of 0.028 g ha^{-1} for Garlon RTU and 0.020 g ha^{-1} for Garlon XRT) were treated as having a concentration of 0 g ha^{-1}. Statistical analyses were completed and plotted using the R software (version 3.6.1) (R Core Team 2019).

Triclopyr residues in leaves from a basal bark treatment of Garlon RTU in *P. tremuloides* saplings were evaluated at ten time points over the year (n = 5 per time point, apart from 59 DAT where n = 4). Translocation of the herbicide from the base of the *P. tremuloides* sapling stem into the leaves took approximately two days to reach maximum concentration, so dissipation was modelled from day two onward. Herbicide concentrations were natural log transformed and a linear model was fit to the data. The model was examined to confirm assumptions of homoscedasticity of model variance (Non-Constant Variance Test, $p>0.05$) and normality of residuals (Shapiro Wilk Normality Test, $p>0.05$). Due to low explanatory power of the linear model, dissipation times were not estimated for basal bark application. Triclopyr residues in *S. bebbiana* leaves from a foliar treatment of Garlon XRT were evaluated at ten time points over the year (n = 5 per time point). Herbicide concentrations were natural log transformed. A hockey stick linear regression consisting of two sequential first order kinetic models with the breaking point estimated at 16.13 days best described the data. The breaking point was determined using the optimization function that searched the data range for the optimal breaking point that minimized the deviance of the fitted model and finds the maximum likelihood estimates. The model was examined to confirm assumptions of homoscedasticity of model variance (ncvTest, $p>0.05$) and normality of residuals (Shapiro Wilk Normality Test, $p>0.05$). Statistical analyses were completed

and plotted using the R software (version 3.6.1) (R Core Team 2019). Dissipation of triclopyr residues was determined for both the time it took residues to dissipate to 50% of the initial concentration 50% (DT_{50}) and the time it took residues to dissipate to 10% of the initial concentration (DT_{90}) for *S. bebbiana* leaves. The DT_{50} and DT_{90} were determined using two sequential first order kinetic equations that defined the hockey stick model as described by the European Union pesticide guideline document (FOCUS 2006):

Equation 4.1

$$C_t = -k_1 t + C_0, \qquad if\ DT < t_{bp}$$

Equation 4.2

$$C_t = (C_0)\left(e^{-k_1 t_{bp}}\right)\left(e^{-k_2 (t - t_{bp})}\right), \qquad if\ DT \geq t_{bp}$$

where C_t is the herbicide concentration in leaves at time t, k_1 is the dissipation rate constant of the kinetics model that described dissipation before the model breaking point (t_{bp}), C_0 is the herbicide concentration at time zero, and k_2 is the dissipation rate constant of the kinetics model that described dissipation after the model breaking point.

4.3. RESULTS

4.3.1. *Drift*

Drift of Garlon RTU (triclopyr) applied at 5230 g a.i. ha^{-1} using the basal bark technique was low, with the maximum concentrations detected falling seven-fold below the allowable application rate. Herbicide was observed immediately in front of and behind *P. tremuloides* sapling stems and at 0.3 m and 0.7 m downwind. Significantly higher triclopyr concentrations were observed at the front and back of the stem compared with 0.3 and 0.7 m, which were not significantly different from each other (Figure 4.6). The highest concentration and most variable concentrations occurred behind the stem (214 ± 50 g a.i. ha^{-1}), followed by in front of the stem (59 ± 21 g a.i. ha^{-1}). Low triclopyr concentrations were observed at 0.3 and 0.7 m (9 ± 3 and 3 ± 1 g a.i. ha^{-1}, respectively). At 0.7 m, 40% of samples had triclopyr concentrations below 1 g a.i. ha^{-1}.

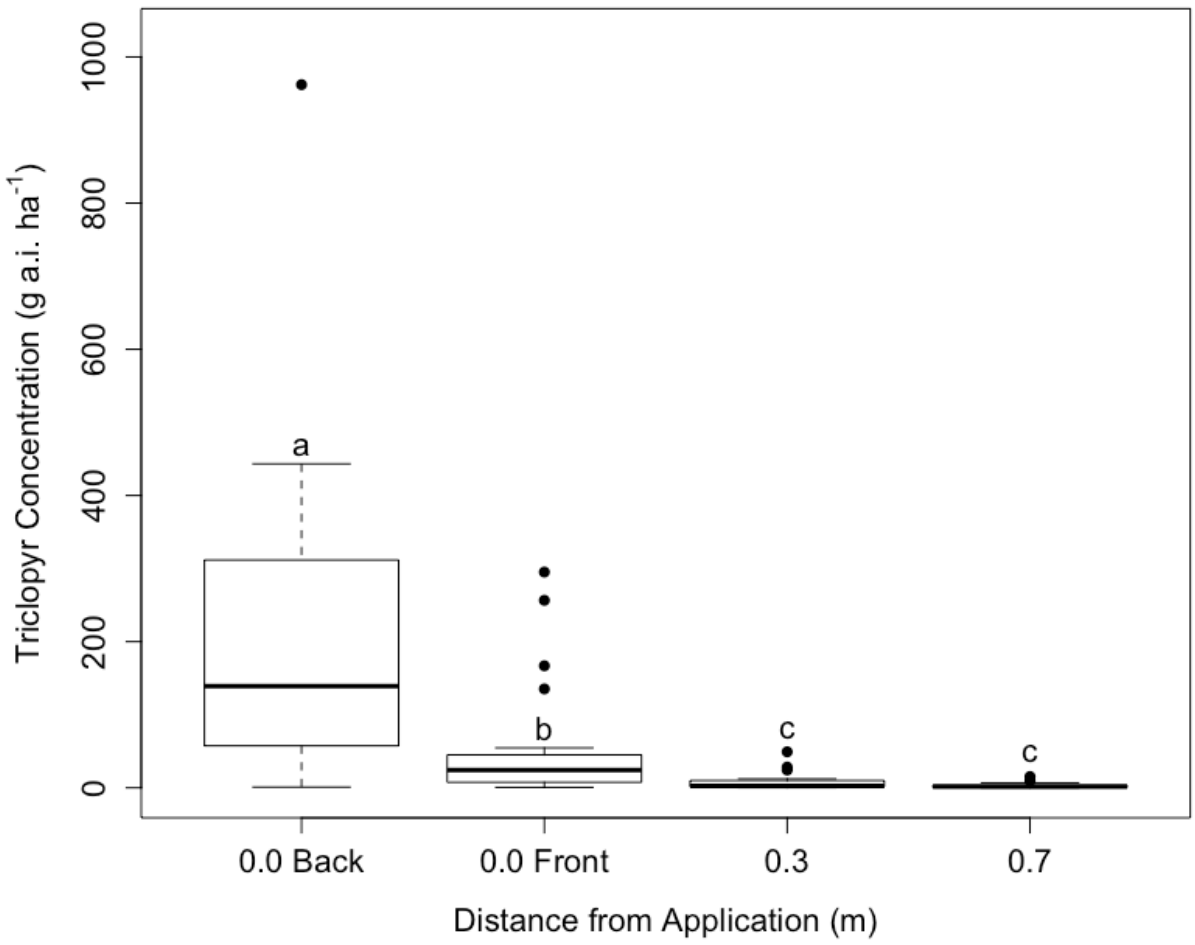


Figure 4.6. Triclopyr drift concentrations observed at four distances from site of application following a basal bark treatment of Garlon RTU (5230 g a.i. ha^{-1}). The mean triclopyr concentration and standard error at the back of the stem was 214 ± 50 g a.i. ha^{-1} (n = 20), 59 ± 21 g a.i. ha^{-1} (n = 20), at the front of the stem and 9 ± 3 g a.i. ha^{-1} (n = 20) and 3 ± 1 g a.i. ha^{-1} (n = 19) at 0.3 m and 0.7 m from the stem, respectively. Different letters indicate a significant difference between distances (ANOVA, TukeyHSD<0.05).

Drift concentrations of triclopyr from low-volume foliar application were overall very low, with the maximum values detected 895 times below the maximum allowable application rate (4530 g a.i. ha^{-1}). Drift from Garlon XRT (triclopyr) applied using a low-volume foliar application was observed immediately beside the stems, and at 0.5, 1.0, and 2.0 m from the site of application. A decrease in mean concentration was observed as the distance from the stem increased, with significantly lower triclopyr concentrations observed at 2.0 m (Figure 4.7). The highest mean concentration and most variability occurred immediately beside the stem (1.3 ± 0.2 g a.i. ha^{-1}) closely followed by 0.5 m from the stem (1.2 ± 0.3 g a.i. ha^{-1}). Concentrations were very low at

1.0 and 2.0 m from the stem (0.6 ± 0.1 g a.i. ha^{-1} and 0.1 ± 0.1 g a.i. ha^{-1}, respectively) and 40% of samples at 2 m were below quantification limit (0.03 g a.i. ha^{-1}). All samples at 2.0 m, 80% at 1.0 m, 60% at 0.5 m, and 45% of samples beside the stem were below 1 g a.i. ha^{-1}.

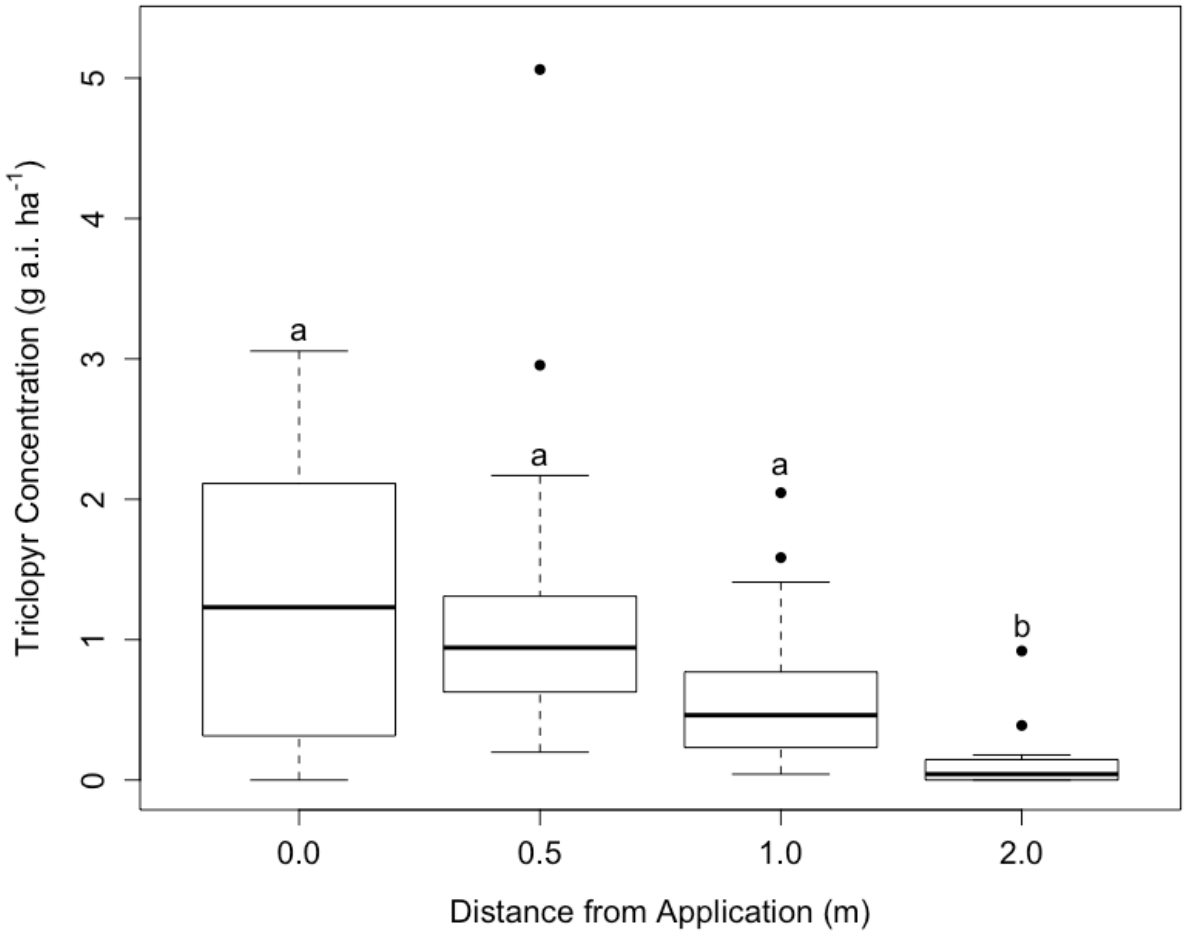


Figure 4.7. Triclopyr drift concentrations observed at four distances from site of application following a low-volume foliar treatment a of Garlon XRT (5540 g a.i. ha^{-1}). The mean triclopyr concentration and standard error (n = 20) at the base of the stem was 1.3 ± 0.2 g a.i. ha^{-1}, and 1.2 ± 0.3 g a.i. ha^{-1} at 0.5 m, 0.6 ± 0.1 g a.i. ha^{-1} at 1.0 m, and 0.1 ± 0.1 g a.i. ha^{-1} at 2 m from the stem. Different letters indicate a significant difference between distances (Kruskal-Wallis, Dunn's test <0.05).

4.3.2. *Dissipation*

Dissipation in leaves of *P. tremuloides* saplings treated with Garlon RTU (triclopyr) applied using the basal bark application followed first order dissipation kinetics (Figure 4.8). Triclopyr dissipation was influenced by the rate of herbicide translocation, metabolism, and dissipation. As a result, high variability was observed in the dissipation data and the linear regression had a

relatively low coefficient of determination ($r^2 = 0.26$) (Figure 4.8). Dissipation times were not estimated for basal bark application due to low explanatory power of the linear model. Approximately two days were required for the herbicide to translocate into the leaves reaching the maximum mean concentration of 204 ± 70 ug g^{-1} (Table 4.1). Concentrations were relatively stable until 14 DAT (197 ± 59 ug g^{-1}), dropping to 57 ± 9 ug g^{-1} at 28 DAT. Concentrations remained at this level through the winter months (between 91 DAT and 280 DAT), and dropped to 10 ± 3 ug g^{-1} a year following herbicide application. Dissipation was also modelled from two days until before the winter break (91 DAT) in Appendix C. No TCP was detected in *P. tremuloides* leaves.

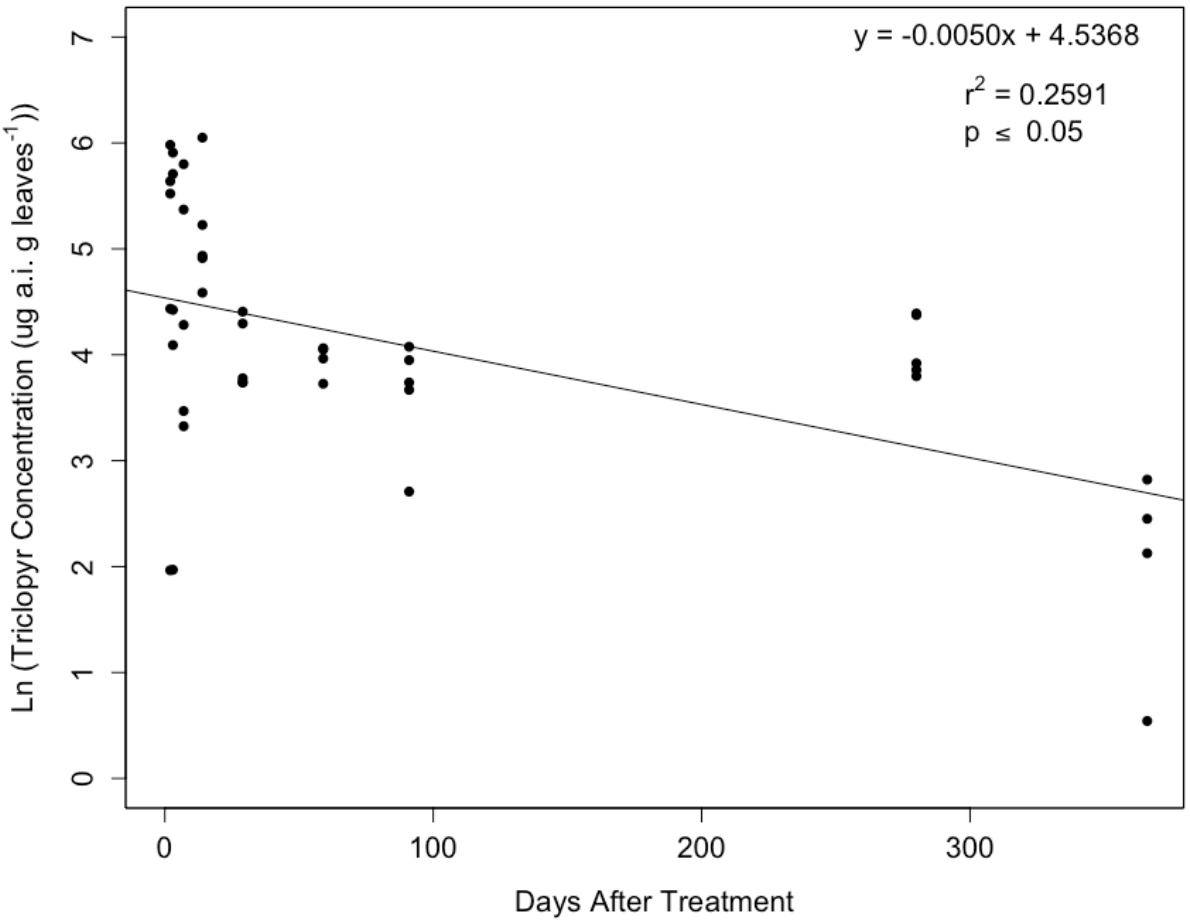


Figure 4.8. First order dissipation model for triclopyr dissipation in the leaves of *P. tremuloides* saplings following a basal bark treatment of Garlon RTU ($r^2 = 0.26$). There was an estimated two-day lag period required to allow for herbicide translocation from the base of the stem into the leaves.

Table 4.1. Mean triclopyr concentration values (and standard errors) in the leaves of *Populus tremuloides* saplings following a basal bark treatment of Garlon RTU. Approximately two days were required for the herbicide to translocate from the base of the sapling stems into the leaves.

Days After Treatment	0	1	2	3	7	14	29	59	91	280	366
Concentration (ug a.i. g^{-1})	15 ± 15	11 ± 4	203 ± 70	164 ± 72	136 ± 59	197 ± 59	57 ± 9	52 ± 4	41 ± 8	61 ± 8	10 ± 3

Dissipation in leaves of *S. bebbiana* shrubs treated with Garlon XRT (triclopyr) via low-volume foliar application followed a hockey stick model consisting of two sequential first order dissipation kinetics. The degradation constants (k_1 = -0.1230, k_2 = -0.0066, SE = 0.6336, p = ≤ 0.05) obtained from the linear regression equation were used to determine the DT_{50} and DT_{90} values as per the equations 4.1 and 4.2, respectively (Figure 4.9). The DT_{50} occurred before the model break point of 16.1 days and was determined to be 5.7 days. The DT_{90} occurred after the model break point at 34.6 days. The maximum mean concentration detected two hours following herbicide application was 1817 ± 245 ug g^{-1}. A year after herbicide application, only 17 ± 2.5 ug g^{-1} was detected in leaves. Dissipation was also modelled from 0 to 90 DAT and 280 to 365 DAT, before and after winter, respectively in Appendix C. No TCP was detected in *S. bebbiana* leaves.

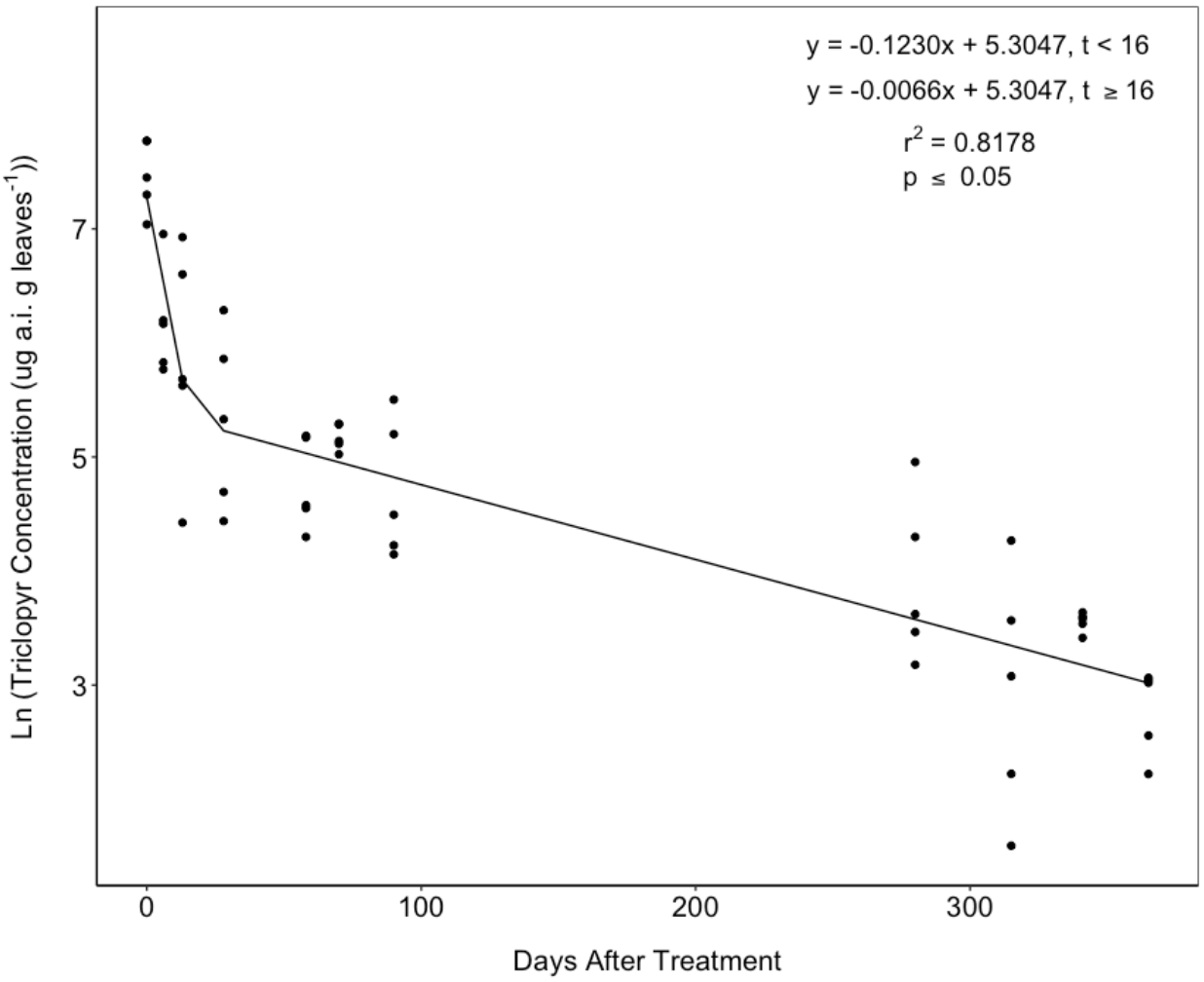


Figure 4.9. Hockey stick model defined by two sequential first order kinetics for triclopyr dissipation in the leaves of *Salix bebbiana* shrubs following a low-volume foliar treatment of Garlon XRT (r^2 = 0.818). The breaking point time of the hockey stick model was determined to be 16.1 DAT. The DT_{50} and DT_{90} were calculated as 5.7 and 34.6 DAT, respectively.

4.3.3. *Risk Assessment*

Triclopyr treated foliage may be a vector of contamination to browsing wildlife. Two common boreal species that are of community concern found on the northern Saskatchewan I3P transmission ROW are moose (*Alces alces*) and the snowshoe hare (*Lepus americanus*), both of which are known to browse on *P. tremuloides* and *S. bebbiana*. Potential triclopyr toxicity for browsing moose and hare were evaluated by use of hazard quotients (HQ), where HQ = exposure concentration / reference toxicity concentration. If the HQ is less than one, the likelihood of risk is acceptable and if the HQ is greater than one, the likelihood of risk is unacceptable. Tier-one HQ

calculations are in Table 4.2 and have been adapted from Jimmo (2018). The acute reference toxicities for moose and hare were extrapolated from LC_{50} values in rats and rabbits, respectively and the chronic toxicity reference was extrapolated from NOEL in a two-generation reproductive rat study (with a safety factor of 100) (Stewart 1991; EPA 1998). The acute risk if these animal species were to browse on litter with the maximum average concentration of triclopyr following basal bark and low-volume foliar applications of Garlon is acceptable, as all HQ values are below one. The average chronic concentration exposure in foliage following low-volume foliar was 284.3 ± 47.2 ug g^{-1} (n = 30 for sampling 6, 13, 28, 58, 70, 90 DAT) and 109.6 ± 20.7 ug g^{-1} from the basal bark application (n = 29 for sampling 3, 7, 14, 29, 59, 91 DAT). There does appear to be an unacceptable chronic risk with both application methods as all HQ values were above one when the average daily intake was calculated using an integrated mean concentration in foliage from late summer to early winter. These results should be interpreted with caution as they reflect the worst-case scenario: these animal species browsing solely on treated litter without consideration of herbicide dissipation or animal foraging behaviour. This is especially true for snowshoe hare, as the food ingestion rate is estimated between June and August when ingestion rates are highest.

Table 4.2. Acute and chronic hazard quotients (HQ) calculated using mean concentrations after maximum triclopyr translocation into foliage between zero and two days after treatment (DAT) (acute exposure) and from late summer to early winter (chronic exposure) in *Salix bebbiana* and *Populus tremulodies* leaves following a low-volume foliar and basal bark treatment of triclopyr, respectively. The average daily intake (ADI) rates and toxicological reference values (TRV) for two representative terrestrial species of *Alces alces* (moose) and *Lepus americanus* (snowshoe hare) were used where, HQ is the ratio of ADI to TRV. If HQ < 1 = acceptable risk and HQ > 1 = likelihood of risk is unacceptable. The HQ values should be interpreted with caution as the use observed concentration for chronic exposure provides the worst-case scenario, without consideration of herbicide dissipation and foraging behaviour.

Herbicide Treatment	Animal Species	Food Ingestion Rate*	Observed Concentrations†		Average Daily Intake		Acute (Mean Max)		Chronic (Integrated Mean)	
			Acute	Chronic	Acute	Chronic	TRV‡	HQ	TRV‡	HQ
		(g dw kg^{-1} bw day^{-1})	(mg ai g^{-1})		(mg ai kg^{-1} bw day^{-1})		(mg ai kg^{-1} bw day^{-1})		(mg ai kg^{-1} bw day^{-1})	
Low-Vol. Foliar – *S.bebbiana*	*A. alces*	37.4	1.8	0.28	67.7	10.6	630	0.11	0.05	**212.7**
	L. americanus	28.4	1.8	0.28	51.6	8.1	550	0.01	0.05	**161.5**
Basal Bark – *P.tremuloides*	*A. alces*	37.4	0.2	0.11	7.6	4.1	630	0.01	0.05	**82.0**
	L. americanus	28.4	0.2	0.11	5.8	3.1	550	0.01	0.05	**62.3**

* Food ingestion rate for *Alces alces* obtained from Renecker & Hudson (1985) for digestible dry matter intake averaged over a year. Food ingestion rate for *Lepus americanus* obtained from Worker et al. (2015) for digestible dry willow sp. intake (assuming lick soil intake) averaged over June and August.

† Acute observed triclopyr concentration for *Salix bebbiana* treated with low-volume foliar application of Garlon XRT from average on day zero of herbicide application and chronic integrated mean from 6, 13, 28, 58, 70, 90 DAT, n = 30 (second sampling point to last sampling point before winter, averaging to 84.3 ± 47.2 ug g^{-1}). Acute observed triclopyr concentration for *Populus tremuloides* treated with basal bark application of Garlon RTU from average on day two of herbicide application, when maximum concentrations were reached and chronic integrated mean from 3, 7, 14, 29, 59, 91 DAT, n = 29 (sampling point after maximum triclopyr translocation into foliage to last sampling point before winter, averaging to 109.6 ± 20.7 ug g^{-1}).

‡ Toxicological Reference Values for triclopyr established on the LD_{50} for female rats (acute) for *Alces alces* and LD_{50} for rabbits (acute) for *Lepus americanus* and the NOEL (5 mg kg $bw^{-1}day^{-1}$) from a two-generation reproduction toxicity study in rats with a safety factor of 100 (chronic) (Stewart 1991; EPA 1998). TRVs specific to ruminants were not available in the literature.

Escalating to a tier-two assessment provides more realistic toxicity exposure assumptions that will indicate if the chronic risk observed from the worst-case scenario in the tier-one assessment is warranted. An environmental impact statement from between 1985 and 1988 provided vegetation data that spans 457 km ROW from Key Lake to Island Falls, Saskatchewan including a width of 10 km centered on the proposed ROW. The I3P ROW located in this surveyed area has an average width of 0.09 km. The summary of vegetation cover from the assessment is outlined in Table 4.3. Moose prefer marsh, mixed woods and deciduous habitats, while hare also occupy these habitats, prefer dense conifer stands in the winter (Feierabend & Kielland 2014). Burn areas were included as suitable habitat for both species as the vegetation cover study was performed decades prior. The suitable habitat data used in the tier-two assessment was determined using a habitat suitability index and aerial moose surveys as determined by Beak Associates Consulting Ltd. (1989).

Table 4.3. Vegetation in the area of the I3P ROW obtained from the Island Falls to Points North 138 kV Transmission Environmental Impact Statement Report prepared by Beak Associates Consulting Ltd. for SaskPower in 1989. Vegetation data was compiled from five Landsat Multi-Spectral Scanner satellite images from 1985 to 1988 of the 456.6 km ROW with a width of 10 km surrounding the proposed ROW. Table adapted from Beak Associates Consulting Ltd. (1989).

Right-of-Way	**Island Falls – Points North, SK (km^2)**
Lakes	818.8
Bogs and Marshes*†	171.4
Bare Sand	73.9
Burn Deciduous*†	29.6
Deciduous Dominate Forest*†	46.5
Deciduous Dominate Mixedwood*†	96.5
Coniferous Dominate Forest†	1729.7
Mature Open Coniferous Forest†	35.6
Regenerated Burn*†	1104.6
Sparsely Vegetated Burn*†	460.0
Total Area (km^2)	**4566.6**
Total Suitable *Alces alces* Habitat (km^2)	**1908.6**
Total Suitable *Lepus americanus* Habitat (km^2)	**3673.9**

* Suitable browse for *Alces alces* used for relative area in determining daily average intake of triclopyr.

† Suitable browse for L*epus americanus* used for relative area in determining daily average intake of triclopyr

Suitable surrounding habitat, residency time, and foraging habits on ROWs for moose and hare were incorporated into chronic average daily intake rates for the tier-two HQ risk assessment (Equation 4.3 and 4.4, Table 4.4). The predation risk of an open corridor within the first year when there is reduced forage and cover can discourage ROW browsing (Joyal et al. 1984; Ricard & Doucet 1999; Feierabend & Kielland 2014; Semeniuk et al. 2014). If suitable forage is available, it is more likely that wildlife will limit their time on the ROW, preferring to browse on the ROW edge and adjacent forest. The occupancy of moose on herbicide treated ROWs was three times less than untreated ROWs and their use of ROW ecotones was approximately three times more than on ROWs (Joyal et al. 1984). Moreover, there was only evidence of moose browsing on the ROW less than 50% of the time they occupied it. This data was extrapolated to hares as they are also known to prefer the area adjacent to open corridors, especially when vegetation cover is lacking as it is on a ROW within the first year of treatment (Joyal et al. 1984; Feierabend & Kielland 2014). When suitable surrounding habitat, ROW residency time, and foraging habits are taken into account, there is only unacceptable chronic risk from a moose browsing on leaves treated with a low-volume foliar treatment of Garlon XRT (Table 4.4). Nevertheless, because of the lack of site-specific information regarding moose and hare browsing habits in the area of the I3P transmission ROW, the tier-two risk assessment is somewhat speculative.

Equation 4.3

$$relative\ suitable\ area\ = \frac{area\ of\ the\ ROW}{total\ area\ of\ suitable\ browse\ 10\ km\ centered\ on\ ROW -\ area\ of\ the\ ROW}$$

Equation 4.4

average daily intake =
(food ingestion rate) x (observed mean concentration) x (relative suitable area) x (predicted frequency on ROW)

Table 4.4. Chronic hazard quotients (HQ) calculated using the integrated mean concentration from late summer to early winter in *S. bebbiana* and *P. tremulodies* foliage following a low-volume foliar and basal bark treatment of triclopyr, respectively. The average daily intake (ADI) rates and toxicological reference values (TRV) for two representative terrestrial species of *A. alces* (moose) and *L. americanus* (snowshoe hare) were used where, HQ = (food ingestion rate x observed mean concentration x relative suitable area x predicted ROW frequency) / TRV. If HQ < 1 = acceptable risk and HQ > 1 = likelihood of risk is unacceptable.

Herbicide Treatment	Animal Species	Food Ingestion Rate* (g dw kg^{-1} bw day^{-1})	Chronic (Late Summer to Early Winter Integrated Mean Concentration)					
			Integrated Mean Concentration† (mg ai g^{-1})	Relative Suitable Area‡ (km^2 / km^2)	Predicted ROW Frequency§	Average Daily Intake (mg ai kg^{-1} bw day^{-1})	TRV| mg ai kg^{-1} bw day^{-1})	HQ
Low-Vol. Foliar	*A. alces*	37.4	0.28	0.02	1/3	0.07	0.05	**1.42**
S.bebbiana	*L. americanus*	28.4	0.28	0.01	1/3	0.03	0.05	0.54
Basal Bark	*A. alces*	37.4	0.11	0.02	1/3	0.03	0.05	0.57
P.tremuloides	*L. americanus*	28.4	0.11	0.01	1/3	0.01	0.05	0.25

* Food ingestion rate for *Alces alces* obtained from Renecker & Hudson (1985) for digestible dry matter intake averaged over a year. Food ingestion rate for *Lepus americanus* obtained from Worker et al. (2015) for digestible dry willow sp. intake (assuming lick soil intake) averaged over June and August.

† Integrated mean concentration from *Salix bebbiana* following low-volume foliar application of Garlon XRT (triclopyr) averaged from 6, 13, 28, 58, 70, 90 DAT (second sampling point to last sampling point before winter, 284.3 ± 47.2 ug g^{-1}). Integrated mean concentration from *Populus tremuloides* treated with basal bark application of Garlon RTU (triclopyr) averaged from 3, 7, 14, 29, 59, 91 DAT (sampling point after maximum triclopyr translocation into foliage to last sampling point before winter, 109.6 ± 20.7 ug g^{-1}).

‡ Area of I3P transition ROW running from Key Lake to Points North, SK is 456.7 km in length by an average of 0.09 km width, totalling 41.1 km^2. This was compared to the area of suitable habitat 10 km surrounding the ROW, of which there was 1908.6 km^2 of suitable moose habitat and 3673.9 km^2 of suitable hare habitat (Beak Associates Consulting Ltd. 1989).

§ Moose use herbicide treated ROWs three times less than a untreated ROW (0.9 ± 1.6 vs. 3.0 ± 2.8 moose/100 km^2, respectively) and occupy the edge of the ROW approximately three times more than the ROW (6.5 ± 4.9 x 2 to account for both edges vs. 4.8 ± 3.6 moose/100 km^2, respectively), while in comparison only browsing on the ROW 2.1 ± 2.5 moose/100 km^2 (Joyal et al. 1984). This data was extrapolated to hare as they are also known to prefer the adjacent area to on open corridor, especially when vegetation cover is lacking as it is on a ROW within the first year of treatment (Joyal et al. 1984; Feierabend & Kielland 2014).

| Toxicological Reference Values for triclopyr established on the NOEL (5 mg kg $bw^{-1}day^{-1}$) from a two-generation reproduction toxicity study in rats with a safety factor of 100 (chronic) (EPA 1998). TRVs specific to ruminants were not available in the literature.

4.4. DISCUSSION

In this study, triclopyr residues from drift and in foliage following basal bark and low-volume foliar treatments to *P. tremuloides* and *S. bebbiana*, respectively were examined under field conditions. Overall triclopyr concentrations from drift following the low-volume foliar application were minimal, whereas much higher concentrations were observed following basal bark application that were localized immediately behind the stem. Conversely, maximum concentrations in foliage following the low-volume foliar treatment were much higher in comparison to that detected following the basal bark treatment, which also required about two days to translocate into the leaves. However, dissipation was rapid from both application methods and triclopyr in leaves was less than 20 ug g^{-1} a year following application. Moose or hare browsing on maximum triclopyr concentrations in leaves from either application technique are unlikely to experience acute toxic effects; however, long-term browsing on contaminated leaves could result in chronic consequences.

4.4.1. *Drift*

Different herbicide application techniques have distinct environmental impacts. It is well known that targeted low-volume herbicide applications have less drift and fewer off-target impacts than high-volume techniques. Nowak and Ballard (2005b) reported herbicide drift depositions averaging 7.3 m for a high-volume foliar application. Drift from hydraulic ground sprayers can cause off-target narcosis within 2 m but that can extend up to 6 m depending on the herbicide (Marrs et al. 1989). Drift from low-volume foliar application in our study was evaluated up to 2 m from the site of application at which distance 40% of samples were below our quantification limit (0.03 g a.i. ha^{-1}). Our results suggest that drift from backpack foliar application is less than those previously found for foliar application by Nowak and Ballard (2005b). Basal bark drift was evaluated to 0.7 m from application at which distance 40% of samples had triclopyr concentrations below 1 g a.i. ha^{-1}. Similarly, Nowak & Ballard (2005b) found relatively low herbicide concentrations from drift at 0.6 m following basal bark applications. The overall low drift concentration detected from our low-volume foliar application resulted in less triclopyr deposition than from our basal bark application. This outcome does not support previous findings that suggest basal bark application deposit less overspray to adjacent non-target plants compared to foliar applications (Dreyer & Niering 1986; Nowak & Ballard 2005b).

Although basal bark techniques have been associated with less drift, higher concentration of herbicide can be localized around individual stems. Depending on the active ingredient concentration in the herbicide mix, this can equate to 100s to 1000s of times more active ingredient per area from basal bark compared to low or high-volume foliar applications; and any adverse effects can be exacerbated in areas with increased stem density (Nowak & Ballard 2005b; Holmes & Berry 2009). Basal bark application of Garlon RTU deposited 165 times more triclopyr at the base of the stem than low-volume foliar. The study by Nowak & Ballard (2005b) found basal bark distributed six times more herbicide than high-volume foliar, and 68 times more than low-volume foliar per treated tree. Although the local herbicide deposition from basal bark application was higher than low-volume foliar, the elevated concentration does not necessarily translate to adverse ecosystem effects.

Non-target vegetation surrounding the stem of the target species was cleared in our study to detect maximum drift concentrations at ground level; however, under normal field conditions decreasing concentrations with distance may result in more spatially localized deleterious effects in non-target species that could intercept spray drift. For example, in one study non-target necrotic foliage and death was observed in a radius of 0.41 m for basal bark and 1.26 m for low-volume foliar treatments, while drift was detected out to 0.6 m and 3.7 m, respectively (Nowak & Ballard 2005b). This was more than was observed by another study, where overspray damage from basal bark was infrequent and only within a 0.25 m radius and within a 1 m radius from a foliar treatment (Dreyer & Niering 1986). In our study, the highest mean triclopyr concentration from drift was detected at the stem base for both targeted application techniques and these values were generally below terrestrial toxicity endpoints outlined in triclopyr registration documents from Agriculture Canada and the U.S. EPA (EPA 1988; Stewart 1991). Concentrations at the base of the stem were also below 28-day LC_{25} and EC_{25} reproduction of boreal invertebrates reported in Jimmo (2018). Moreover, the maximum mean concentrations detected in our study were 1.4 times below the most sensitive terrestrial invertebrate toxicity endpoint of 25% *F. candida* triclopyr avoidance for basal bark application and 260 times below for low-volume foliar application (see Chapter 5, Section 5.4.4). However, the highest drift concentrations detected (2.38 ± 0.56 ug g^{-1}, converted from area concentration using soil density from a La Ronge, SK study (Holmden & Bélanger 2010)) did fall above the 50% growth inhibition concentration (IC_{50}) (1.59 ± 0.74 ug g^{-1}) for fireweed (*Chamerion angustifolium*) germination, the most sensitive non-target terrestrial boreal plant tested (Isbister et

al. 2017). Typically signs of injury on non-target plants are found at a greater distance than lethal effects and the herbaceous community often completely recovers within two years following application (Marrs et al. 1989; Newmaster et al. 1999; Isbister et al. 2017). Drift was detected at the furthest distances evaluated for basal bark (0.7 m) and low-volume foliar (2 m) applications, but concentrations were relatively low and unlikely to cause lasting off-target toxicity.

Low-volume foliar and basal bark herbicide treatments applied via backpack sprayer are both targeted application methods that select and treat individual undesired ROW species. Typically, low-volume foliar treatments deposit less herbicide, but with further drift. Whereas, basal bark treatments are associated with higher herbicide concentrations localized at the base of the stem and less drift. For these reasons, low-volume foliar applications are used in areas with higher stem density when off-target herbicide deposition is less of a concern and basal bark applications are used when stem density is lower, so as not to exacerbate local adverse effects, and when non-target deposition is less acceptable (Nowak & Ballard 2005b; Holmes & Berry 2009). Our results support these management practices, but also agree with previous studies that triclopyr concentrations regardless of targeted application method are unlikely to cause lasting adverse non-target effects (Marrs et al. 1989; Newmaster et al. 1999; Isbister et al. 2017).

4.4.2. *Dissipation*

The differences in application technique between low-volume foliar and basal bark herbicide applications result in very different concentrations in foliage. Maximum concentrations were not reached until about two days following basal bark application and concentrations were approximately nine times lower than the maximum concentration observed immediately following the low-volume foliar treatment. The herbicide following a basal bark treatment undergoes absorption, translocation, and metabolism before maximum concentrations are detected in foliage and triclopyr concentrations were therefore much less and more variable than what was measured on foliage from low-volume foliar. Despite drastically different maximum concentrations, triclopyr residues in foliage from both application methods were comparable one year following application (basal bark, 17 ± 2.5 ug g^{-1} and low-volume foliar 10 ± 3 ug g^{-1}). More rapid dissipation following low-volume foliar compared with basal bark could be the result of unabsorbed triclopyr on the leaf exterior being removed by rainfall, dew, volatilization, photolysis, and microbial degradation. The lower maximum herbicide concentration in foliage following basal bark

techniques makes it advantageous over low-volume foliar applications when toxicity to browsing animals or herbicide movement into water bodies following leaf abscission is of concern.

There is limited information regarding the interaction of herbicides and boreal vegetation, resulting in uncertainty as to how herbicide sensitivities compare to more southern and widely tested crop species (Princz et al. 2012). Generally, triclopyr dissipation rates have been relatively rapid in most species, which is consistent with our findings for boreal species. Triclopyr residues identified in *S. bebbiana* foliage treated via low-volume foliar application of Garlon XRT were examined in central Saskatchewan (54 °N latitude). Our triclopyr DT_{50} from *S. bebbiana* of 5.7 days was below dissipation rates seen in willow (*Salix glauca*) in the Yukon Territory (64 °N latitude) of 11.5 days (Jimmo et al. 2018). Triclopyr DT_{50} values were just over six days of boreal species (Douglas fir (*Pseudotsuga menziesii*), mountain lover (*Paxistima myrsinites*), Wood's rose (*Rosa woodsii*), sticky current (*Ribes viscosissimum*), and snowberry (*Symphoricarpos albus*)) in an Idaho (44^{o} latitude) study using a foliar application (Whisenant & McArthur 1989). In Alaska (59 °N latitude), triclopyr concentrations were low or undetectable in the foliage of salmonberry (*Rubus spectabilis*), false azalea (*Menziesia ferruginea*), blueberry (*Vaccinium ovalifolium*), high-bush cranberry (*Viburnum edule*), and red elderberry (*Sambucus racemosa*) 30 - 45 DAT (Newton et al. 2008). Our DT_{90} was observed within this time range (34.6 DAT), however our concentrations (and application rate) were much higher. There have been few studies that focus on herbicide residues in boreal species following basal bark applications. In our study an average concentration of 41 ug g^{-1} was observed at 91 DAT following a basal bark application to *P. tremuloides*. Despite being treated by low-volume foliar, similar dissipation was observed in Alaska (65 °N latitude) with the same aspen species 120 DAT (23 ug g^{-1} to 55 ug g^{-1}) when initial detected concentrations (~390 ug g^{-1}) were also similar (Newton et al. 2008). Comparison of triclopyr dissipation in foliage between different boreal species is summarized in Table 4.5.

Table 4.5. Comparison of the dissipation of maximum detected triclopyr concentrations in the foliage of boreal species.

Species	Lat °N*	Max. Triclopyr conc. (ug g^{-1})	Triclopyr Conc. (ug g^{-1})			Study
			< 14 d†	30-45 d	91-121 d	
Willow (*S.bebbiana*)	54	1817	DT_{50} (5.7 d)	34.6		Ours
Willow (*S. glauca*)	64	137	DT_{50} (11.5 d)			Jimmo et al. (2018)
Douglas fir, mountain lover, Wood's rose, sticky current, and snowberry	44	151 - 79	DT_{50} (~6 d)			Whisenant & McArthur (1989)
Salmonberry, false azalea, blueberry, high-bush cranberry, and red elderberry	59	~320		Low to ND‡		Newton et al. (2008)
Aspen (basal bark)	54	204			41	Ours
Aspen (foliar)	65	~390			23 - 55	Newton et al. (2008)

* Northern latitude (°N)
† Days (d)
‡ Non-Detect (ND)

Comparing triclopyr dissipation across studies is challenging because of variations between experimental designs and species used that can also be confounded by environmental factors. The longer dissipation seen in our study may be influenced by higher application rates and therefore greater initial concentrations; however, that is not always the case. Even at similar application rates, our average initial concentration (1817 ug g^{-1}) following low-volume foliar was 14 times higher than those seen in a similar species of Yukon willow (*S. glauca*) (137 ug g^{-1}), yet initial dissipation rates were comparable (Isbister 2016). The relatively low temperature at the time of foliar application (14 °C) may have reduced initial triclopyr dissipation on leaves through volatilization, photolysis, and microbial breakdown, resulting in higher concentrations of triclopyr being absorbed. Despite these differences it appears that even at the higher application rates or initial concentrations, such as those in our study, relatively rapid dissipation (DT_{50} <14 DAT) of

triclopyr occurs in boreal plant species. The influence of vegetation species and environmental conditions on triclopyr dissipation kinetics needs to be more fully understood by conducting further studies on more northern and naturally occurring species.

4.4.3. *Risk Assessment*

A tiered HQ risk assessment using extrapolated interspecies toxicity endpoints was implemented to evaluate the risk associated with moose and snowshoe hare browsing on triclopyr contaminated foliage following low-volume foliar or basal bark applications. Tier-one risk assessments incorporate conservative exposure assumptions more indicative of a worse-case scenario, whereas tier-two assessments integrate better defined exposures specific to the scenario providing more realistic toxicity assumptions (Meek et al. 2011). The HQ results of the tier-one assessment indicate an acceptable acute risk (extrapolated from LC_{50} in rats and rabbits) if a moose or hare were to browse on foliage with the maximum average concentration of triclopyr following low-volume foliar or basal bark applications of Garlon (1817 ug g^{-1} immediately following treatment and 204 ug g^{-1} 2 DAT allowing for translocation, respectively). However, with both herbicide application techniques there does appear to be an unacceptable chronic risk (extrapolated from the NOEL in a two-generation reproduction rat study with a safety factor of 100) when using the mean foliage concentrations from late summer to early winter (284.3 ± 47.2 ug g^{-1} low-volume foliar and 109.6 ± 20.7 ug g^{-1} basal bark treatment). It is reasonable to assume that increased predation risk on ROWs and available suitable habitat adjacent to the I3P ROW would discourage boreal herbivores from exclusively browsing on the treated ROW (Joyal et al. 1984; Ferron & Ouellet 1992; Hodson et al. 2010; Van Beest et al. 2010; Semeniuk et al. 2014). When both relative suitable habitats surrounding the ROW and predicted browsing frequency on the ROW are considered, the chronic risk becomes acceptable for hares, but remains unacceptable for moose. Foraging behaviour on ROWs are multifactorial and challenging to anticipate and therefore it is important to understand the context and limitations of the available information.

Rights-of-way can promote movement and foraging on the landscape, especially since they are maintained as early - to mid - successional habitats that provide preferred browsing foliage for many animals (Bramble & Byrnes 1982; Meilleur et al. 1994; Rempel et al. 1997; Semeniuk et al. 2014; Pattison & Catterall 2019). However, increased ROW browsing is typically observed if adjacent foraging is poor or there is adequate vegetation cover on the ROW to seek refuge from

predators (Ricard & Doucet 1999; Rieucau et al. 2009; Semeniuk et al. 2014; Andersen et al. 2017; Mumma et al. 2019). White-tail deer browsed less on ROWs even when stems were more abundant, except when that abundance exceeded six times the surrounding area (Mayer & Carvell 1975; Bartzke et al. 2014). The ecotone created between the ROW and adjacent forest can provide both adequate cover from predators and desirable forage (Bramble & Byrnes 1979; Joyal et al. 1984; Ferron & Ouellet 1992; Bartzke et al. 2014). More tracks and pellets of deer and moose have been reported in ecotones than in adjacent open corridors (Bartzke et al. 2014). Moose have been observed occupying the edge of ROWs in the winter approximately three times more than on ROWs, while only browsing within ROWs 44% of the time they occupied it (Joyal et al. 1984). Moreover, moose use herbicide treated ROWs approximately three times less than untreated ROWs, while only browsing within treated ROWs 20% of the time they occupied it (Joyal et al. 1984). Hare too have resided more in ecotones and prefer feeding in areas with greater herbaceous vegetation density and canopy closure than in open habitats to facilitate escape from predators (Ferron & Ouellet 1992; Hodson et al. 2010; Hodson et al. 2011). Furthermore, in the summer moose select quality forage over quantity and in the winter avoid areas with minimal canopy that would increase snow depth and thus would unlikely solely browse on treated ROWs if the adjacent habitat was suitable (Joyal et al. 1984; Peckarsky et al. 2008; Van Beest et al. 2010). Therefore, as suitable habitats are available adjacent to the ROW, it is unlikely that wildlife would exclusively browse on the treated I3P ROW with reduced browse quality and cover from predators.

Foraging behaviour of wildlife on anthropogenic linear features are hard to predict as it is a complex relationship of balancing daily energetic requirements, minimizing energy loss, and considering predation risk (Semeniuk et al. 2014). Escalating to a tier-two assessment with more realistic toxicity exposure assumptions is difficult with the limited availability of information surrounding boreal animal species' use of ROWs and associated browsing habits. The suitable habitat data used in the tier-two risk assessment was established from a habitat suitability index, which relies on expert knowledge on the capacity for a habitat to support a species. Resource selection function is the superior method in determining utilized habitat. This method is based on empirical data that inherently integrates the contribution of multiple factors involved in wildlife habitat selection (Osko et al. 2004). Although the tier-two HQ calculated in this study provides a more realistic estimate of risk to browsing wildlife, site-specific data would improve confidence in the current assessment.

4.4.4. *Conclusion*

In this study, triclopyr residues from drift and in boreal foliage following basal bark and low-volume foliar treatments to *P. tremuloides* and *S. bebbiana*, respectively were examined under field conditions in boreal Saskatchewan. Drift from these targeted applications is spatially limited with elevated concentrations observed at the base of stems, especially from basal bark. Nevertheless, herbicide concentrations were below levels of ecological concern based on previously determined endpoints for boreal soil invertebrates and common boreal plants, apart from germination of fireweed (basal bark concentration behind the stem $>IC_{50}$). Triclopyr dissipation in boreal foliage was observed to be relatively rapid, especially in foliage treated by low-volume foliar with concentrations that were initially much higher than from basal bark application. This may be the result of unabsorbed triclopyr on the leaf surface immediately following foliar application, whereas any triclopyr detected in foliage treated via basal bark was within the leaf. Triclopyr concentration in foliage from the treatment methods were similar and less than 20 ug g^{-1} one year following treatment, despite very different maximum concentrations.

Targeted applications of triclopyr present limited drift (40% of basal bark samples at 0.7 m and all foliar samples at 2 m were less than 1 g a.i. ha^{-1}), relatively rapid dissipation in foliage (DT_{50} for foliar was 5.7 days and below 60 ug a.i. g^{-1} at 30 DAT for basal bark), and an acceptable level of acute and chronic risk to browsing hares and acute risk to browsing moose. Site-specific data regarding browsing behaviour on ROWs, especially where herbicide has been applied are needed to improve confidence in the tier-two risk assessment. Basal bark applications may be a preferable treatment where toxicity to browsing animals is of concern, but deleterious effects to soil ecosystem could arise in areas with high stem density.

PREFACE: CHAPTER FIVE

There is uncertainty regarding the indirect effects of triclopyr on habitat quality. Litter decomposition is essential for soil nutrient cycling and ultimately ecosystem health. Herbicides that indirectly enter the soil ecosystem upon leaf abscission could influence these processes by altering litter chemistry or impacting decomposer species. The indirect effects of triclopyr on habitat quality were examined through litter mass loss and quality (carbon:nitrogen ratios), as well as the response of boreal invertebrates (*Folsomia candida* and *Oppia nitens*) in microcosms and avoidance tests. The results of this study will provide novel information and a more comprehensive understanding on the influence of triclopyr on habitat quality in boreal ecosystems.

There is redundancy in the information provided in the background and literary review of Chapter One to provide context and support for the results of the experiments in this chapter.

Voinorosky C., Stewart K. Environmental Impact of Triclopyr on Habitat Quality in Northern Saskatchewan

Chelsea Voinorosky: Conceptualization, methodology, investigation, data curation, validation, analysis, writing (original draft), visualization
Katherine Stewart: Conceptualization, analysis, writing (review and editing), supervision, project administration, resources, funding acquisition

5. ENVIRONMENTAL IMPACT OF TRICLOPYR ON HABITAT QUALITY IN NORTHERN SASKATCHEWAN

5.1. INTRODUCTION

The impact of herbicides on terrestrial ecosystems has been primarily investigated in agricultural environments (Wagner et al. 2004; Rüegg et al. 2007; Bhagirath et al. 2017) and temperate climates (Meilleur et al. 1994; Wagner et al. 2004; Rombke et al. 2006b; Newton et al. 2008; Barnes & Seefeldt 2009). Far fewer examinations of herbicide toxicity have occurred in boreal ecosystems and of these investigations direct toxicity to plants, soil invertebrates, and wildlife have been the focus (Wagner et al. 2004; Rombke et al. 2006b; Newton et al. 2008; Clark et al. 2009). A comprehensive understanding of the impacts of herbicides in boreal systems requires a better understanding of the potential indirect effects on the system via habitat quality. Herbicides can indirectly enter the soil ecosystem upon leaf abscission following translocation into foliage. This can cause direct toxicity to litter decomposers and impact litter decomposition rates and nutrient release critical for ecosystem health. Plant litter decomposition and mineralization facilitated by soil invertebrates and microorganisms are essential in the formation of soil organic matter and soil fertility (Aber & Melillo 1980; Carcamo et al. 2001). Litter decomposition directly contributes to habitat quality by providing nutrients that support flora and fauna (Hall et al. 1997).

The herbicide triclopyr mimics the plant growth hormone, auxin, which inhibits senescence processes that translocate nutrients into belowground rhizomes before winter and consequentially nutrients remain in treated foliage (Poovaiah 1974; Gottschalk & Shure 1979; Brown 1997). Moreover, auxin can indirectly inhibit lignification in leaves resulting in less lignin in foliage that is taxing for microorganisms to metabolize (Cromack & Monk 1975; Linck 1976; Gottschalk & Shure 1979; Austin & Ballare 2010). Ultimately, treated foliage tends to have higher nutrient concentrations, including nitrogen (lower C:N) and less lignin that could result in increased decomposition and nitrogen mineralization compared to that of untreated foliage (Gottschalk & Shure 1979). Elevated nitrogen content and associated lower C:N ratios have been observed in herbicide treated litter (Gottschalk & Shure 1979) and these changes may directly influence decomposition rates (Taylor et al. 1989; Tian et al. 1992; Kim 2007; Zhang et al. 2008).

Initial stages of organic matter decomposition are stimulated by climate, litter nutrient concentration, and the species and abundance of decomposing organisms (Couteaux et al. 1995;

Berg 2000; Gergocs & Hufnagel 2016). The inability to remove confounding environmental factors can result in complications when interpreting responses to contaminants like herbicides (Siciliano & Roy 1999). Mass loss of leaf litter in early stages of decomposition is largely associated with the leaching of water-soluble compounds and the presence of critical nutrients that support microbial activity (Couteaux et al. 1995; Berg 2000; Naiman et al. 2005; Gergocs & Hufnagel 2016). Degrading organisms have a high demand for nitrogen and therefore it is often the limiting nutrient in leaf decomposition rates (Manzoni et al. 2008). Low C:N profiles in leaf litter have been associated with increased mass loss and nitrogen mineralization in early stages of litter decomposition (Bryant et al. 1998; Berg & McClaugherty 2003; Naiman et al. 2005; Kim 2007; Laganiere et al. 2010).

Soil and litter dwelling invertebrates contribute to soil fertility through litter fragmentation and digestion which supports microbial decomposition and nutrient cycling processes. Litter decomposition from arthropod contribution increased rates by an average of 23% according to 15 reviewed studies with half of those studies reporting arthropods increased nitrogen mineralization rates as well (Seastedt 1984). Although several studies have focused on the ecological effects of herbicides like triclopyr, there have been few studies published specific to boreal ecosystems examining the response of critical native microflora and fauna to herbicides (Braune et al. 1999; Gamberg et al. 2005; Rombke et al. 2006b; Princz et al. 2012). The invertebrate profiles in boreal regions differ from other ecosystems. Northern soil faunal compositions have lower species diversity than in temperate regions and well-studied macrofauna like earthworms, important to litter fragmentation in temperate regions, are scarce or absent in northern climates (Behan 1978; Behan-Pelletier 1997; Sjursen et al. 2005; Loureiro et al. 2009). Mesofauna like Collembola, Acari, and Enchytraeidae are critical contributors to organic matter decomposition in northern ecosystems with Collembola and mites comprising 95% of total microarthropods (Seastedt 1984; Carcamo et al. 2001; Rombke 2003; Sjursen et al. 2005). Mesofauna assist in litter fragmentation, which increases surface area for efficient microbial-facilitated metabolism and creates a favorable environment through deposition of nutrient rich fecal material into the soil (Cambardella 2005).

Boreal mesofauna have been reported to be relatively insensitive to direct herbicide exposure. Few herbicide toxicity studies have been conducted with mites, but adverse effects to Enchytraeids and Collembola from herbicides like triclopyr, phenmedipham, atrazine, and trisulfuron were

observed above maximum allowable application rates in these studies (Sabatini et al. 1998; Novais et al. 2010; Jimmo et al. 2018). Toxicity to decomposers may impair ecosystem services by changing spatial and temporal nutrient release dynamics. Moreover, the effects of triclopyr on habitat quality may be consequences of indirect effects to invertebrates induced by herbicides.

Avoidance tests can provide a realistic indicator of habitat quality that is reflective of field conditions. Although the underlying mechanism of avoidance behavior is not well understood, soil invertebrates can use olfactory and gustatory chemoreceptors to detect and potentially avoid contaminants before adverse effects (Gainer et al. 2019a). Avoidance responses are typically sensitive, rapid, and an important complement to existing ecotoxicological tests (Loureiro et al. 2005a; Bori & Riva 2015). However, avoidance tests are not always more sensitive than sublethal endpoints. Collembola are attracted to volatile odours from organic matter and fungus, and have displayed an attraction response to some contaminants like hydrocarbons (Bengtsson et al. 1988; Wenke et al. 2010; Gainer et al. 2019b). Terrestrial invertebrate avoidance tests in soil have been standardized for springtail (*Folsomia candida*) and earthworms (*Eisenia fetida* and *Eisenia andrei),* but to our knowledge standardized tests for contaminated leaf litter have not yet been developed (ISO 2008, 2011). Habitat quality compromised by herbicides may result in the avoidance of treated leaf litter, decreasing leaf litter decomposition rates and impacting boreal ecosystem health.

The use of the herbicide triclopyr has been proposed for vegetation management on northern rights-of-way (ROWs). Earlier research suggests that direct ecological impact in terrestrial systems is relatively minimal. Maximum herbicide application rates for Garlon XRT (triclopyr) were below the concentration in soil that reduced reproduction rates by 25% (EC_{25}) for common boreal soil invertebrates (*F. candida, Oppia nitens*, and *Enchytraeus crypticus*). However, there is still uncertainty regarding the indirect effects of these herbicides on habitat quality (Clark et al. 2009). The fitness of litter degrading organisms relates to habitat quality, which is subject to leaf litter and soil properties. In this study, the indirect effects of the herbicide Garlon XRT to habitat quality were examined via boreal litter breakdown rates, litter quality, and the response of important decomposer boreal mesofauna. The first objective of these experiments was to assess the impact of Garlon XRT at maximum field application rates on litter mass loss (breakdown rates) and C:N (litter quality) by use of field and laboratory litterbag trials. The second objective was to evaluate

the response of boreal mesofauna (*F. candida* and *O. nitens*) to Garlon XRT. This was achieved by comparing reproduction rates of mesofauna (*F. candida* and *O. nitens*) residing in treated or untreated boreal leaf litter contained in laboratory microcosms over four months. Additionally, 48-hour avoidance tests were performed to determine if each mesofauna species would avoid treated leaf litter. Establishing the influence of triclopyr on litter breakdown rates, C:N profile, and boreal decomposers will improve our understanding of the effects of herbicide application on habitat quality and is critical for responsible integrative vegetation management (IVM) on northern ROWs.

5.2. METHODS

5.2.1. *Leaf Breakdown in Field Litterbags*

Breakdown of willow (*Salix bebbiana*) leaf litter was examined at the Willow site employing the previously described study design (Section 4.2.1, 4.2.2. (4.2.2.3), and 4.2.3, Figure 4.4). Leaves on the untreated *S. bebbiana* shrubs had naturally senesced and begun abscission by 70 days (October 10, 2017). Leaves from 20 treated and 24 untreated *S. bebbiana* shrubs were collected from the Willow site 70 days following the low-volume foliar application of Garlon XRT, which occurred on August 2, 2017. The experiment was also conducted 28 days following herbicide application before *S. bebbiana* leaves on the control shrubs had senesced and begun abscission and is described in Appendix D. Treated leaves and untreated leaves were compiled into two composite samples, respectively. An equal mass of *S. bebbiana* leaves from treated or untreated composites (8 ± 0.01g) were placed in 1.3 mm^2 nylon mesh litterbags (15 x 15 x 1 cm) (Chomel et al. 2016). Since drying can influence herbicide residue and provide unrealistically low moisture in the leaves, litter was not dried prior to placement in the litterbags. Five additional 8 ± 0.01g subsamples from collected treated and untreated foliage at 70 days after treatment (DAT) were dried at 60 °C for 72 hours and reweighed to determine the initial dry mass conversion at time of litterbag burial**.**

In total, 32 litterbags with untreated leaves and 32 litterbags with treated leaves were buried in the untreated control area and limited by the available leaves on the control shrubs, only 32 litterbags with treated leaves were buried in the treated area 70 DAT (time zero). In the untreated control area, litterbags were buried at a depth of 5 cm (Kozlov & Zvereva 2015) around the perimeter of untreated *S. bebbiana* shrubs. Litterbags were buried alternating between treated and

untreated with a minimum of 2 m between treated and untreated litterbags. In the treated area, litterbags were buried in the same manner at the perimeter of treated *S. bebbiana* shrubs.

Eight randomly selected litterbags from each treatment were recovered at 280, 315, 341, and 365 DAT. Upon recovery, litterbags were stored individually in Ziploc bags and transported in a cooler with icepacks to the laboratory. The litter was brushed of soil particles, exogenous organic matter, and visible soil invertebrates (Lucisine et al. 2015). For each treatment (treated and untreated in control area and treated in treated area) litter from five litterbags was weighed, oven dried at 60 °C for 72 hours, and reweighed to determine mass loss (Yang et al. 2005). All mass values for determination of mass loss were corrected to dry weight. Mass loss comparison was determined according to the Organization for Economic Cooperation and Development guidance document on the breakdown of organic matter in litterbags using the following formula (OECD 2006):

Equation 5.1

$$\% \text{ mass loss} = \frac{\text{start weight-end weight}}{\text{start weight}} \times 100$$

Total carbon and total nitrogen were determined by dry combustion for a subset of samples from both field and microcosm litterbags. Field litterbag samples were freeze-dried, and microcosm litterbag samples were oven-dried prior to homogenization using a ball-grinder (Retsch MM-400, Germany). Weights of approximately 0.50 g of litter were recorded before combustion at 1350 °C with the TruMac CNS analyzer (LECO, USA). Samples were analyzed with four standards of Leco Orchard Leaves (502-055; Carbon 50.4% ± 0.4, Nitrogen 2.28 ± 0.04%) with an average of (n = 4) 50.40 ± 0.00 for carbon and 2.28 ± 0.01 for nitrogen and wheat flour as a quality control every ten samples with an average (n = 10) of 43.95 ± 0.3 for carbon and 2.38 ± 0.01 for nitrogen.

5.2.2. *Laboratory Microcosms*

5.2.2.1. *Material Collection*

Materials for the microcosm and avoidance tests were harvested from the La Ronge site 10 km north of La Ronge on September 20, 2017 (Appendix A). Leaves in the area were

predominantly naturally senesced and had begun abscission. Approximately 500 g of leaves from each species (i.e., aspen (*Populus tremuloides*), paper birch (*Betula papyrifera*), and willow (*Salix bebbiana*)) were harvested from at least 40 trees or shrubs over an area of approximately 600 m^2. Composite soil samples were taken with a shovel at random to a depth of 5 cm within an area approximately 12 m^2 that contained all three of the plant species sampled. The surface organic horizon (LFH layer) was removed prior to soil collection and approximately 60 kg (wet weight) of soil was collected. Soil samples were air dried in a dark room and sieved to less than 2 mm (Yang et al. 2012). Leaves were stored at -20 °C to kill any soil fauna with the exception of microfauna (nematodes, rotifers, protozoans, and tardigrades) and microarthropod eggs (incomplete mortality) (Lenoir et al. 2007; Yang et al. 2012).

A composite sample of leaf litter (approximately 300 g) was also harvested across the 600 m^2 site to confirm the presence of *Folsomia candida* and *Oppia nitens.* Within three days of collection, the leaf litter was used to extract soil mesofauna. Mesofauna were extracted from leaf litter using modified Berlese-Tullgren funnels in a chamber with string lights (series max of 480 W) that was heated to a temperature of 32 °C (Gergocs & Hufnagel 2016). Fifty funnels were lined with 1.3 mm^2 mesh and filled with 3.0 g of leaf litter. The heat and light encouraged mesofauna escape from the desiccating litter in funnels that were attached above containers lined with plaster of Paris and activated charcoal (5:1). Every day for three days the plaster was moistened and mesofauna were collected from the bottom containers and compiled with like species in separate containers lined with plaster. Mites, including *O. nitens*, were the most abundant mesofauna collected, followed by Collembola, primarily juvenile *F. candida*.

5.2.2.2. *Invertebrate Culturing*

All invertebrate species were cultured in the Soil Toxicology Laboratory at the University of Saskatchewan, Saskatoon, Saskatchewan. Collembola (*F. candida*) were cultured according to standard operating procedures (OECD 2008; Environment Canada 2014). Cultures of *F. candida* were reared in a plastic container with a 5:1 plaster of Paris and activated charcoal bottom and stored in the dark at a temperature of 20 ± 2 °C. Baker's yeast was used as a food source and added with water as needed (every 2 – 3 days). The age of *F. candida* used for study were synchronized by transferring egg clusters from stock cultures into new culture vessels lined with plaster. Two

days following hatching, juveniles were transferred into new vessels where they were cultivated for study when synchronized to an age of 10 - 12 days.

Mites (*O. nitens*) were cultured according to standard operating procedures (Princz et al. 2010). Cultures were reared in a 125 mL glass jar with a base of 5:1 plaster of Paris and activated charcoal and stored in the dark at a temperature of 20 ± 2 °C. Baker's yeast was used as a food source and added with water as needed (every 2 – 3 days). The age of *O. nitens* used for study were synchronized by transferring 20 - 24-day old red adults into new culture vessels lined with plaster for seven days, leaving the juveniles behind. Juveniles were synchronized and ready to be used in microcosm experiments when amber in color, between 30 - 42 days old.

5.2.2.3. *Herbicide Treatment and Invertebrate Inoculation*

Two herbicide treatments were applied: i) maximum application rate to a composite leaf litter sample of *P. tremulodies, B. papyrifera*, and *S. bebbiana* and ii) no herbicide applied to a composite leaf litter sample (Table 5.1). Leaves from each species were spread out on a tray in a single layer and sprayed with Garlon XRT at the maximum allowable triclopyr concentration for forest use, 4.53 kg a.i. ha^{-1}. Herbicide was applied to field collected leaves using a custom-built track sprayer from the University of Saskatchewan (Agassiz Scientific Ltd. of Saskatoon, Saskatchewan). The sprayer was calibrated to 218 L ha^{-1} at a speed of 4.5 km hr^{-1} and pressure of 40 psi, a procedure modified from Isbister (2017). The TeeJet 8002E flat fan nozzle was used (Spraying Systems Co., Wheaton, IL) at 50 cm above leaves. Control leaves were sprayed with distilled water by the same method. Between treatments, equipment was washed with soap and water and acetone rinsed. Treated litter was air dried for two hours before placement in litterbags and use in microcosms. In addition, two invertebrate inoculation treatments were examined: i) no mesofauna and ii) mesofauna (Table 5.1). Each invertebrate inoculum treatment was examined under each herbicide treatment for a total of four microcosm treatments replicated eight times. Microcosms were destructively sampled (n = 32) at four time intervals up to four months for a total of 128 microcosms (Yang et al. 2012).

Table 5.1. Microcosm herbicide and decomposer inoculation treatments: Leaf litter treated with foliar application of Garlon XRT (4530 g a.i. ha^{-1}) (H) or untreated (C) was contained in litterbags and placed into microcosms. Microcosms were inoculated with mesofauna (*Folsomia candida* and *Oppia nitens*) (M) or not (N).

Microcosm ID	Decomposer Inoculation	Herbicide Treatment
N – C	No mesofauna	No herbicide (control)
N – H	No mesofauna	Herbicide
M – C	Mesofauna	No herbicide (control)
M – H	Mesofauna	Herbicide

5.2.2.4. *Construction of Microcosms*

Each microcosm was contained in a plastic container (20 cm length x 16 cm width x 10 cm height) and filled with mineral soil, organic soil, and a litterbag (Hattenschwiler & Gasser 2005). To obtain a more homogenous microbial population across the microcosms, the soil was pasteurized in an oven at 105 °C for 24 hours and then inoculated with the following suspension. Approximately 100 g of each litter type (*P. tremuloides, B. papyrifera*, and *S. bebbiana*) and 2 kg of the unpasteurized soil from the La Ronge site was ground and mixed with approximately 7 L of distilled water for five minutes (Yang et al. 2012). The suspension was used to inoculate the pasteurized soil in each microcosm to 60% water holding capacity. An 8 ± 0.01 g equal part mixture of *P. tremulodies, B. papyrifera*, *and S. bebbiana* treated or untreated with herbicide was contained within a 1.3 mm^2 nylon mesh litterbag (15 x 15 x 1 cm). For treatments with mesofauna, twenty synchronized *F. candida* and *O. nitens* were added to the litter. Litterbags were placed over 300 g (dry weight) mineral layer and 100 g (dry weight) of organic layer, where both soil layers were hydrated to 60% water holding capacity. Five additional 8 ± 0.01 g equal part mixtures of *P. tremulodies, B. papyrifera*, and *S. bebbiana* leaves were dried at 60 °C for 72 hours and reweighed to determine the initial dry mass conversion. The microcosm was sealed with a lid (containing holes to allow for gas exchange), weighed, and stored for 1, 2, 3, or 4 months at 20 ± 2 °C with a 16-hour light and 8-hour dark photoperiod (60% humidity). Soil moisture in the microcosms was maintained weekly at 60% water holding capacity by adding water with a fine-mist spray bottle to maintain the initial weight. The pH of the soil and litter was measured at time of microcosm construction and at each destructive sampling. Prior to the initiation of the experiment, a pilot study was conducted for two months using three microcosms of each treatment-inoculation scheme to ensure the above design was adequate.

5.2.2.5. *Microcosm Sampling*

Microcosms were destructively sampled at 1, 2, 3, or 4 months. At each time point, five microcosms of each treatment (Table 5.1) were used to extract the mesofauna and determine litter breakdown and quality. The remaining three microcosms of each treatment were used for herbicide analysis. Mesofauna (*F. candida* and *O. nitens*) were extracted using modified Berlese-Tullgren funnels, as previously described (Section 5.2.2.1). Extracted mesofauna were counted from digital images taken with a Cannon PowerShot ELPH 180 camera (20 MP) using the Image J software (version 1.51s) (Rasband 2018). Following extraction, the litter was further dried at 60 °C for 72 hours, and reweighed to determine the dry mass (Yang et al. 2005). All mass values for determination of mass loss were corrected to dry weight. Mass loss comparison was determined using Equation 5.1 (Section 5.2.1). Litter quality was determined by homogenizing and sub-sampling (~0.5 g) to determine the C:N ratios using LECO TruMac CNS analyzer (LECO Corporation, St. Joseph, MI) as previously described (Section 5.2.1). The remaining leaf litter (~5 g) was used for triclopyr and TCP analysis. The treated litter was stored at -20 °C until analysis of triclopyr and TCP concentrations.

5.2.3. *Triclopyr Avoidance*

5.2.3.1. *Study Design*

Equal parts *P. tremulodies, B. papyrifera*, and *S. bebbiana* collected from the La Ronge site (described previously, in 5.2.2.1 Material Collection) were stored at -20 °C and maintained at field moisture content (40.2 ± 0.9%). Leaves from each species were spread out on a tray in a single layer and sprayed with Garlon XRT at different concentration of triclopyr determined appropriate for each invertebrate from range finding tests. Twelve doses were used for Collembola avoidance (0, 1133, 2265, 3398, 4530, 6795, 9060, 10570, 12080, 13590, 15100, 16610 g a.i. $ha^{-1)}$ and ten doses were used for mite avoidance (0, 2265, 4530, 11325, 13590, 15085, 16625, 18120, 19615, 21155 g a.i. ha^{-1}). Herbicide was applied to field collected leaves using a custom-built track sprayer from the University of Saskatchewan (Agassiz Scientific Ltd. of Saskatoon, Saskatchewan) using a procedure modified from Isbister (2017) as previously described (Section 5.2.2.3). Control leaves were sprayed with distilled water by the same method. Between treatments, equipment was washed with soap and water and acetone rinsed.

Leaves of the same treatment were homogenized in a blender two hours after treatment, sieved up to 4 mm, and hydrated with distilled water back to field moisture by weight. The blender was cleaned with soap and water and acetone rinsed between treatments. Homogenized leaves were mixed with artificial soil in a 1:5 ratio by wet weight. Artificial soil consisted of 10% sphagnum moss (air dried, sieved < 2 mm), 20% kaolin clay, and 70% silica sand that was allowed to equilibrate three days prior to the test, then adjusted to a pH between 6.0 and 7.5 with calcium carbonate (Princz et al. 2010; Environment Canada 2014). Artificial soil was hydrated with water to 70% water holding capacity. Glass jars were divided into two sides separated by a plastic divider with one side of the container containing leaf litter treated with Garlon XRT mixed with artificial soil and the other untreated litter mixed with artificial soil (Table 5.2). An additional dual-control test (negative control) was performed with no herbicide treatment on either side of the container to test for the absence of avoidance reaction. Upon divider removal, ten individual mesofauna of *F. candida* or *O. nitens* were placed on the midline of the container and the container was closed to minimize water loss by evaporation. The avoidance tests ran for 48 hours at a temperature of 20 ± 2 °C with a 16-hour light and 8-hour dark photoperiod. Each dose (12 for Collembola, 10 for mites) was replicated ten times.

Table 5.2. Container dimensions and contents for study of Collembola (*F. candida)* and mites (*O. nitens)* avoidance response to triclopyr. One side of the container contained litter treated with Garlon XRT (triclopyr), while the other side contained untreated litter. Litter (treated and untreated) was mixed with artificial soil in a 1:5 ratio by wet weight.

Invertebrates	Container Dimensions (cm)				Litter Layer per Side (g)	Soil Layer per Side (g)
	Width	Height	Top	Bottom		
F. candida	8.1	6.5	7.3	8.1	2	10
O. nitens	7	6	6	5	1	5

5.2.3.2. *Avoidance Sampling*

Avoidance was observed based on the residency of mesofauna in either the treated or untreated litter after 48 hours. At the conclusion of the avoidance test, the division was reinstated and mesofauna from each section was extracted from the leaf litter artificial soil mixture using modified Berlese-Tullgren funnels (procedure previously described in Section 5.2.2.1). Missing mesofauna were considered dead. A net response was obtained from each test using the following equation (IOS 2006):

Equation 5.2

$$\text{net response (\%)} = \frac{\text{number of organisms on untreated side - number of organisms on treated side}}{\text{total number of organisms recovered}} \times 100$$

A positive net response indicates avoidance and a negative net response indicates attraction to the treatment. Avoidance tests were considered valid if there was less than 20% mortality, the mean net response of negative controls (two untreated sides) was 0 ± 10% (all doses discarded if not met), and standard deviation across replicates of the same dose was less than 30 (IOS 2006).

5.2.4. *Herbicide Analysis*

Refer to Section 4.2.4 Herbicide Analysis for the method of detecting triclopyr from leaf samples and Appendix B for analysis in detail. Refer to Appendix E for measured triclopyr concentrations for avoidance doses.

5.3. Statistical Analysis

Mass loss differences over four time points and between treatments were compared for litter buried in field litterbags 70 DAT (n = 5 per time interval for each treatment). Time and treatment and the interaction of time and treatment were examined using a two-way Analysis of Variance (ANOVA) followed by TukeyHSD post hoc tests ($p<0.05$). Mass loss differences over time and between herbicide treated and untreated litter, either with or without invertebrates (*F. candida*) (n = 5 by 4 treatments) contained within laboratory microcosms were analyzed using three-way ANOVA with interactions followed by TukeyHSD post hoc tests ($p<0.05$).

A subset of three field litter bags per time interval were randomly selected for C:N analysis. Carbon:Nitrogen ratio differences over time and between treatments of litter buried in field litterbags were analyzed using two-way ANOVA followed by a TukeyHSD post hoc test ($p<0.05$) (n = 3). Prior to ANOVA, C:N ratios were natural log transformed. Differences between C:N over time and between herbicide treated and untreated litter, either with or without invertebrates (*F. candida*) (n = 3, for all four treatments with the exception of untreated with invertebrates at 59 and 126 DAT and untreated without invertebrates at 91 and 126 DAT where n = 2) contained within laboratory microcosms were analyzed using three-way ANOVA followed by TukeyHSD post hoc tests ($p<0.05$). Assumptions of equal variance (Bartlett Test of Homogeneity of Variance, $p>0.05$)

and normality for the model residuals (Shapiro-Wilk Normality Test, $p>0.05$) were confirmed for all ANOVA models (mass loss and C:N).

Triclopyr residues in litter buried in field litterbags 70 DAT following foliar application of Garlon XRT were evaluated at five time points (n = 5 at 70 DAT, and n = 6 at 280, 315, 341, and 365 DAT). Litter treated with Garlon XRT contained in laboratory microcosms litterbags was also examined (n = 6 per time interval). A zero-order kinetics model was the best fitting linear regression with the highest coefficient of determination (R^2) for both dissipation models. Homoscedasticity of model variance (ncvTest, $p>0.05$) and normality of model residuals (Shapiro, $p>0.05$) were confirmed.

Collembola numbers over time and between herbicide treated and untreated litter with invertebrates (*F. candida*) (n = 8 for 30 and 59 DAT and n = 7 at 91 DAT for treated and untreated litter, and n = 8 for treated litter at 126 DAT and n = 9 for untreated litter) contained within laboratory microcosms were analyzed using two-way ANOVA with interactions followed by TukeyHSD post hoc tests ($p<0.05$). Collembola juvenile numbers over time and between herbicide treated and untreated litter with invertebrates (*F. candida*) (n = 8 for 30 and 59 DAT for treated and untreated litter) contained within laboratory microcosms were analyzed using two-way ANOVA with interactions followed by TukeyHSD post hoc tests ($p<0.05$). Prior to ANOVA, Collembola adult and juvenile numbers were log transformed to meet the assumption of equal variance (Levene's Test, $p>0.05$) and normality of model residuals were confirmed (Shapiro, $p>0.05$).

Avoidance tests were considered effective if there was less than 20% mortality, the mean net response of negative controls was 0 ± 10%, and standard deviation across same dose replicates was less than 30 (IOS 2006). Tests that did not meet validity were removed from the data set with every dose having at least seven of ten replicates. The avoidance test responses using a concentration of 18120 g a.i. ha^{-1} were removed for the mite data set before analysis due to possible technical error in dosing. The four-parameter Weibull dose response model from the drc package in the R software best described the invertebrate net avoidance responses to triclopyr concentrations (Equation 5.3) (R Core Team 2019). In this model, the lower and upper limits were constrained to 0 (no avoidance) and 100 (complete avoidance), respectively. Models coefficients and associated p-values were assessed for each model. Lack of fit was assessed using the

'modelFit' function, which compares the dose-response model to a one-way ANOVA using an F-test (p-value>0.05). In addition, Akaike's Information Criterion values were compared across a range of models to confirm the best fitting model. The effective triclopyr concentration that caused 25% (EC_{25}), 50% (EC_{50}), and 70% (EC_{70}) avoidance in tested invertebrates and the associated 95% confidence intervals were determined using the 'ED' function. All statistical analyses were completed and plotted using the R software (version 3.6.1) (R Core Team 2019).

Equation 5.3

$$f(x) = c + (d - c)\exp(-\exp\big(b(\log(x) - \log(e))\big)$$

where, b is the steepness of the curve, c is the lower limit of response, d is the upper limit of response, and e is the effective dose.

5.4. RESULTS

5.4.1. *Mass Loss*

Litter mass loss was examined in field litterbags buried 70 days after a low-volume foliar treatment with Garlon XRT (triclopyr) where untreated leaves were buried in the untreated area and treated leaves were buried both in the treated and untreated areas. Mass loss increased over time; however, there were no significant differences in mass loss between treatments, including between treated litterbags buried in the treated and untreated areas (Figure 5.1). No significant difference in mass loss was observed in treated litter between 341 and 365 DAT. Mass loss increased from ~10% at 280 DAT to ~26% one year following treatment.

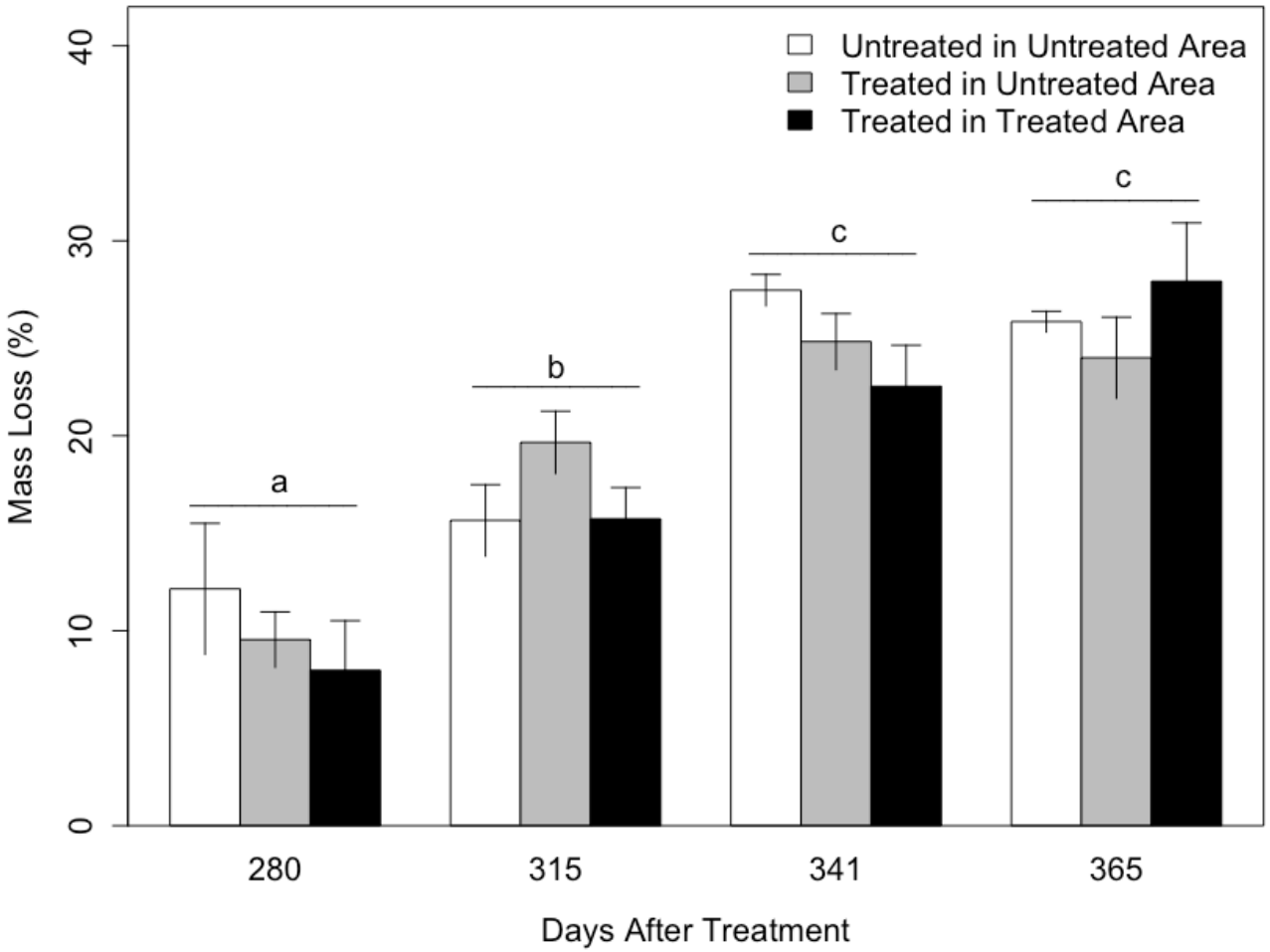


Figure 5.1. Mass loss of willow leaves (*S. bebbiana)* buried in field litterbags at 70 days after a low-volume foliar application of Garlon XRT (triclopyr), where untreated litter was buried in an untreated area and treated litter was buried in both treated and untreated areas. Bars represent mean mass loss expressed as a percentage with standard error and different letters denote significant differences (ANOVA, TukeyHSD<0.05).

Litter mass loss within laboratory microcosm litterbags was examined over time and between herbicide treated (laboratory application of Garlon XRT to uncontaminated leaves collected in the field) and untreated litter, either with or without invertebrates (*F. candida*). Mass loss increased over time; however, mass loss was not significantly different between treated and untreated leaves except for significantly more mass loss in treated leaves (4.4% higher) at 30 DAT (Figure 5.2). The presence of *F. candida* in the microcosms had no significant effect on mass loss in either treated or untreated leaves. Mass loss increased from ~31% at 30 DAT to ~46% at 126 DAT.

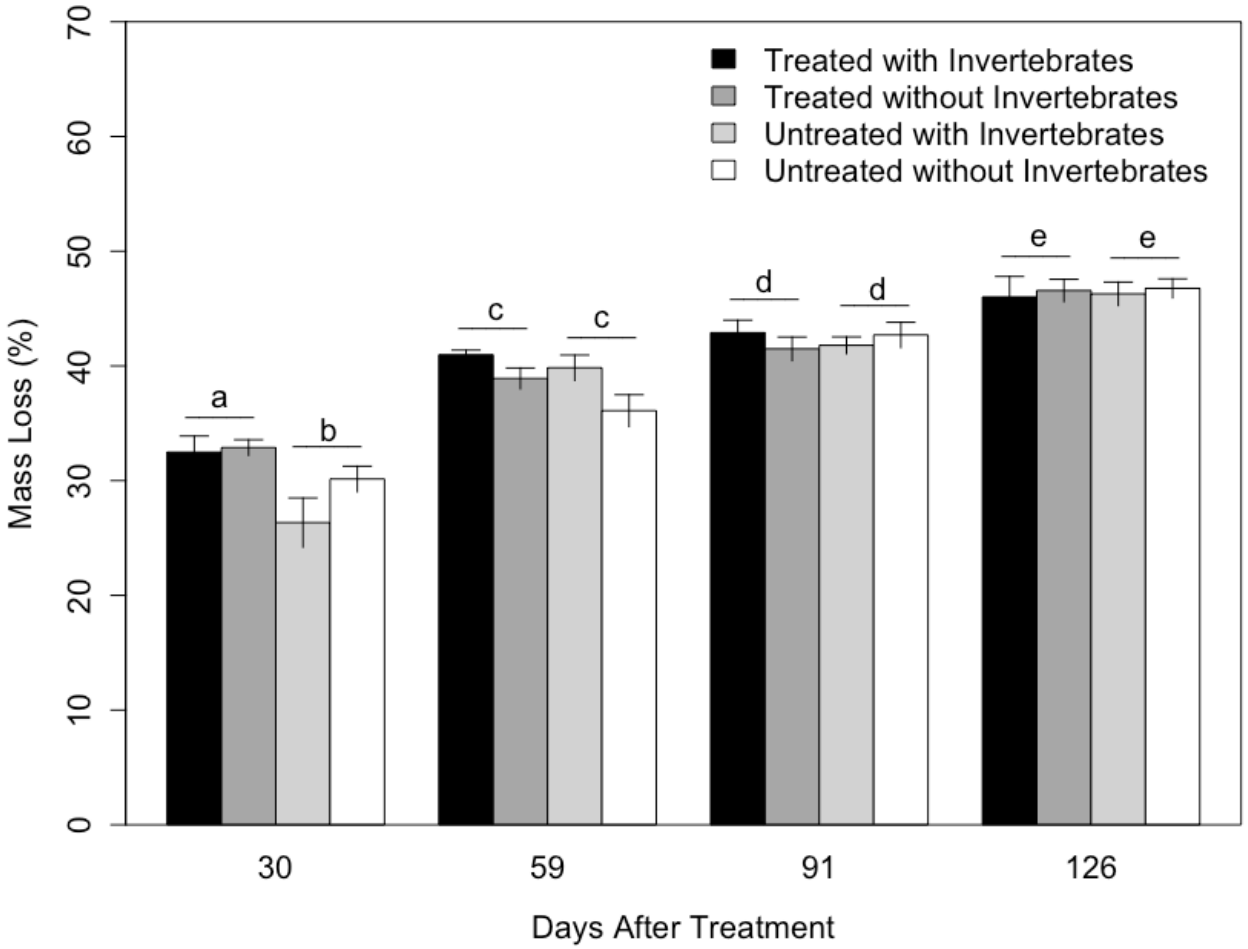


Figure 5.2. Mass loss of *P. tremuloides*, *B. papyrifera*, and *S. bebbiana* leaves contained within litterbags in laboratory microcosms two hours following a low-volume foliar application of Garlon XRT (triclopyr). Bars are means with standard error and different letters denote significant difference between treated vs. untreated litter (excluding invertebrates) and days after treatment (ANOVA, TukeyHSD<0.05).

5.4.2. *Litter Quality*

Carbon:Nitrogen ratios of litter in field litterbags were significantly higher in untreated than treated leaves at all time intervals. At 70 DAT when the litterbags were buried, untreated litter had a C:N of 70.7 ± 0.7 and treated litter of 29.1 ± 0.1. A significant decrease in C:N for both untreated (55.1 ± 3.1) and treated (23.6 ± 0.5) leaves were observed upon the first recovery at 280 DAT (Figure 5.3). Although there was a decreasing trend in C:N ratios over time, C:N ratios were not significantly different from 280 to 365 DAT.

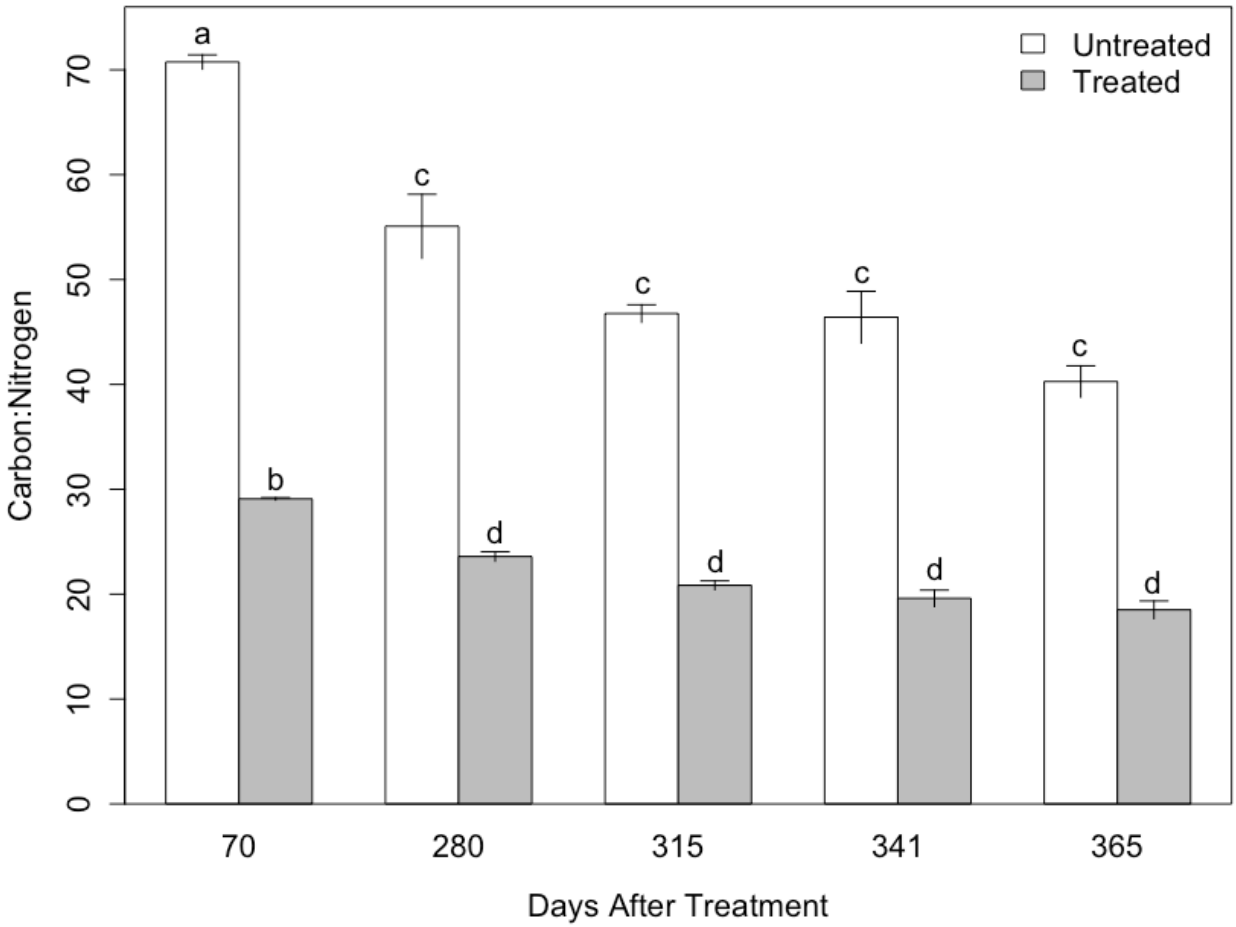


Figure 5.3. Litter quality of *S. bebbiana* leaves buried in the field litterbags at 70 days after a low-volume foliar application of Garlon XRT (triclopyr). Litter quality expressed as carbon:nitrogen ratio recovered at four time points over a year. Bars represent means with standard error and different letters denote significant differences (ANOVA, TukeyHSD<0.05).

There were no significant differences in C:N ratios of litter in laboratory microcosms between any of the treatments. When microcosms were constructed (i.e., time zero), there was no significant difference in the C:N of treated (42.3 ± 0.2) and untreated (41.3 ± 0.2) leaves. The initial C:N in litter was significantly higher than at 30 DAT and 59 DAT (Figure 5.4). The largest decrease in C:N (i.e., ~40 to 25) occurred between the initial litter at time 0 and 30 DAT. However, the observed decrease in C:N was more gradual over the next three months and no significant difference in C:N was observed between 91 and 126 DAT (Figure 5.4).

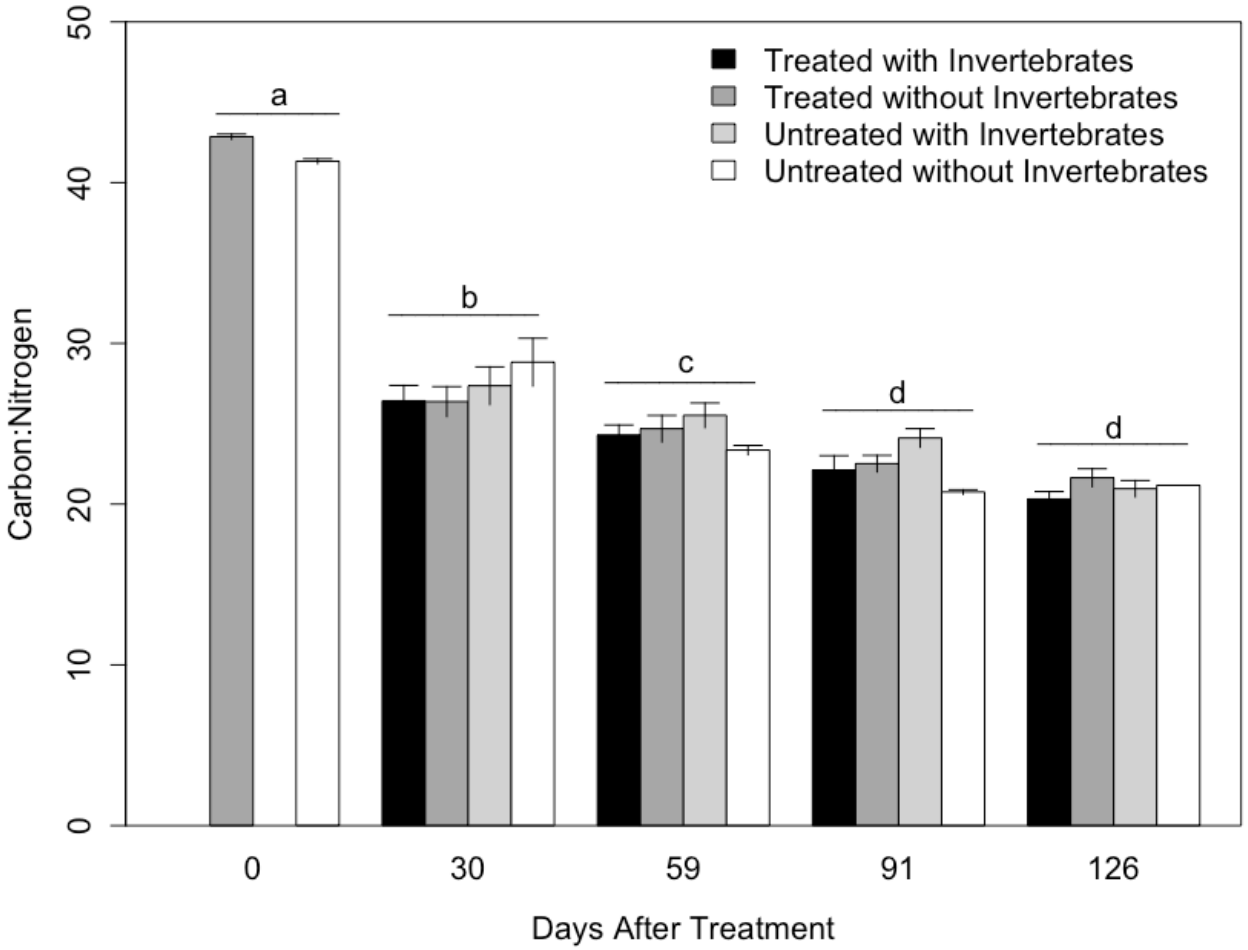


Figure 5.4. Litter quality of a mixture of *P. tremuloides*, *B. papyrifera*, and *S. bebbiana* leaves contained within litterbags in laboratory microcosms two hours following a low-volume foliar application of Garlon XRT (triclopyr). Litter quality expressed as carbon:nitrogen recovered at four time points over a year. Bars represent means with standard error and different letters denote significant difference over time (ANOVA, TukeyHSD<0.05).

The decrease observed in the C:N ratios of litter in both field litterbags and laboratory microcosm litterbags was influenced by increasing nitrogen. In all cases except treated litter in the microcosms, carbon concentrations decreased with time, although not significantly so. Whereas, nitrogen concentrations increased over the duration of the experiment. In the field, nitrogen in untreated litter increased by 68% between 70 DAT and 365 DAT and treated litter increased by 38% between 70 DAT to 365 DAT. This translates to 72% more nitrogen in treated than untreated litter at 70 DAT when litterbags were buried and 107% more at 365 DAT. In microcosms there was only a 6% difference between treated and untreated nitrogen content at both 0 and 126 DAT, although there was still an increase in litter nitrogen over time.

5.4.3. *Triclopyr Residue Dissipation in Litterbags*

Dissipation of triclopyr in treated *S. bebbiana* leaves buried in field litterbags 70 DAT followed zero order dissipation kinetics (Figure 5.5). For comparison, the terminal dissipation phase (70 DAT to 365 DAT) from leaves collected aboveground on *S. bebbiana* shrubs at the same site was modelled. The dissipation rate constant was nearly identical regardless of if leaves were buried in litterbags or still attached to shrubs aboveground (Figure 5.5; k = -0.49 for litter belowground and -0.48 aboveground). Triclopyr concentrations detected at 70 DAT (177 ± 9 ug g^{-1}) were from leaves collected aboveground and used for time zero in field litterbag construction. Overall, triclopyr concentration detected in litterbags and aboveground leaves decreased by 88% and 90% respectively from 70 DAT to one year following herbicide application.

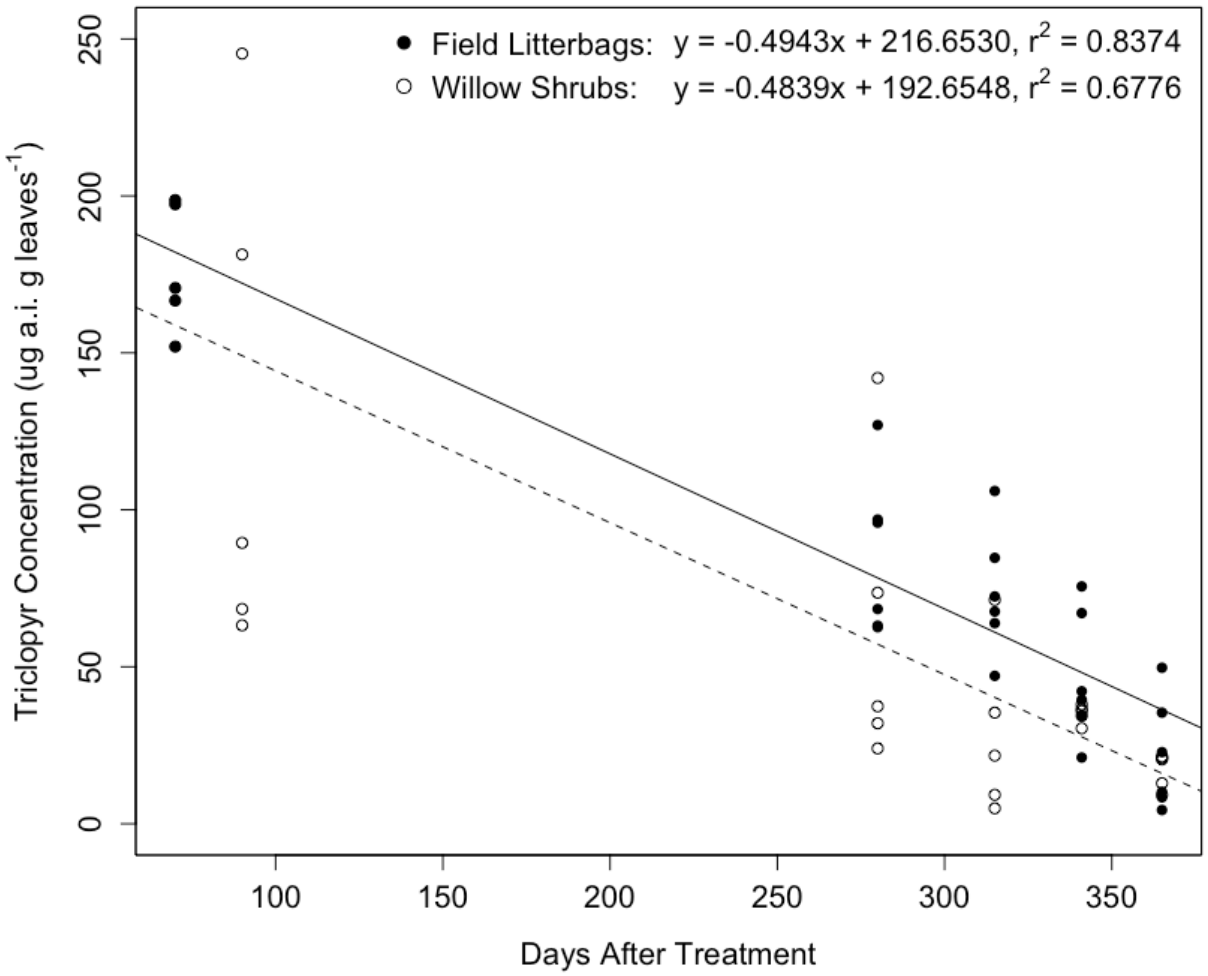


Figure 5.5. Solid black points and trend line depict the zero-order linear regression model for triclopyr dissipation in *S. bebbiana* leaves buried in field litterbags 70 days after treatment with Garlon XRT at five time points ($r^2 = 0.837$). The model emulates that which was observed in above ground *S. bebbiana* leaves following 70 DAT of Garlon XRT (open circles and dashed trend line). Values at 70 DAT were from leaves collected aboveground and used for time zero in litterbag construction.

Dissipation in leaves contained within laboratory microcosms followed zero order dissipation kinetics (Figure 5.6; k = -13, SE = 1.3, p = ≤ 0.05). The mean herbicide concentration in litter used in microcosm litterbags at time zero was 2124 ± 84 ug g^{-1}. The triclopyr concentration detected in microcosm litter decreased by 69% by 91 DAT.

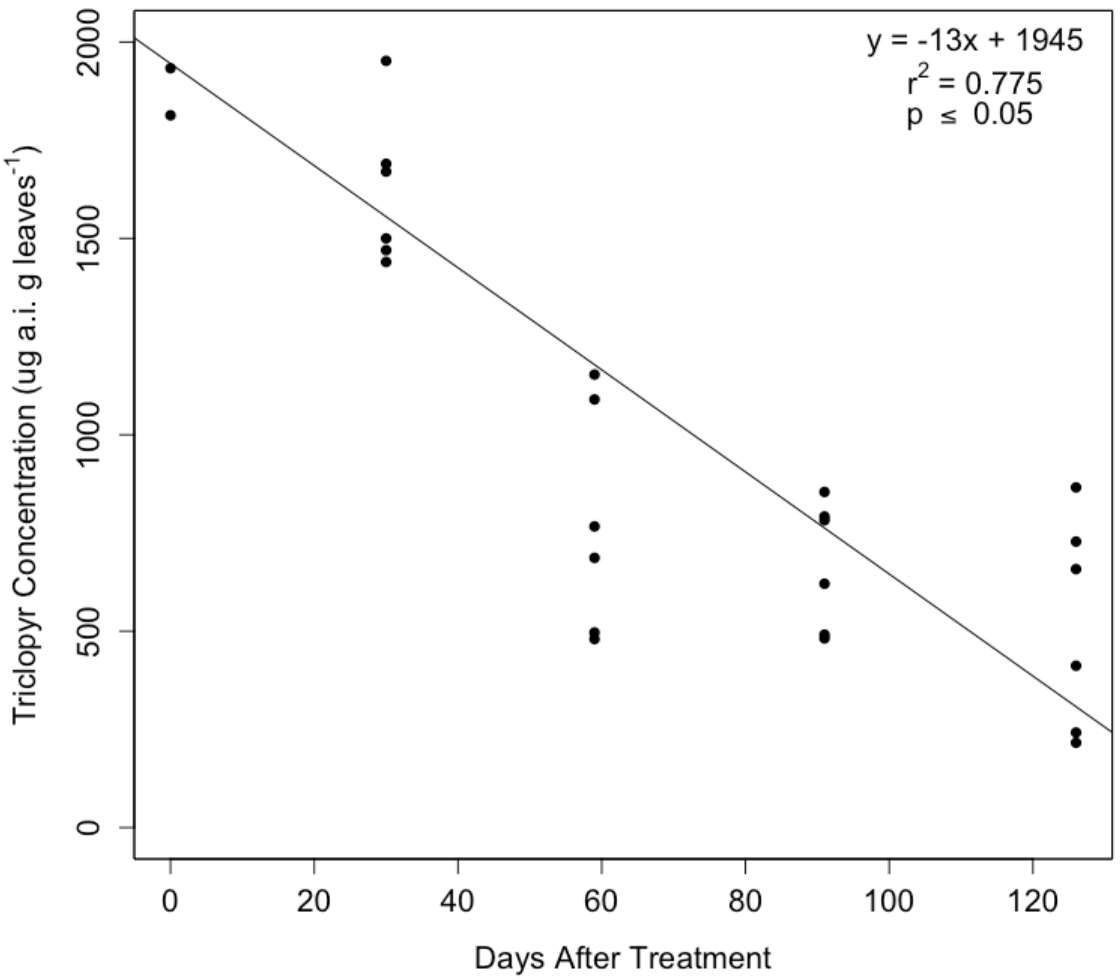


Figure 5.6. Zero order linear regression model for triclopyr dissipation in litter from microcosm litterbags treatment with Garlon XRT at five time points ($r^2 = 0.775$).

5.4.4. *Invertebrate Toxicity*

The total *F. candida* numbers in microcosms were not significantly different between treated and untreated litter at each time point (Figure 5.7). However, *F. candida* numbers were overall higher in untreated litter than in treated litter. There was also no significant difference in *F. candida* juvenile numbers between treated and untreated litter, but numbers were again higher in untreated litter (Figure 5.8). Microcosms were inoculated with *O. nitens* along with *F. candida*; however, mite survival was less than 15% at 30 DAT, and 0% in subsequent sampling periods.

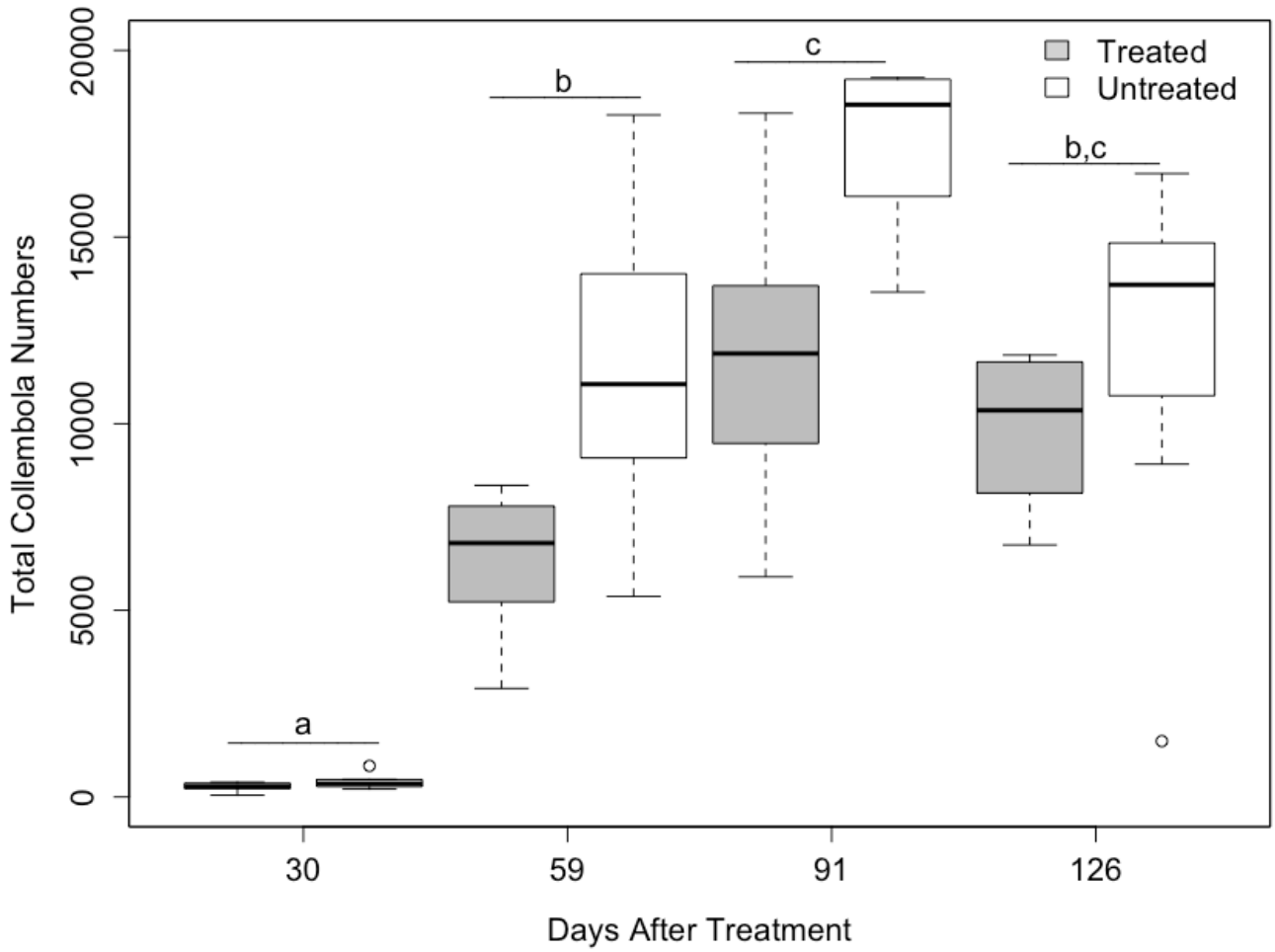


Figure 5.7. The total Collembola (*F. candida*) numbers observed within litterbags in laboratory microcosms up to 126 days following a laboratory treatment of Garlon XRT (triclopyr). Bars represent means with standard error and different letters denote significant difference over time (ANOVA, TukeyHSD<0.05).

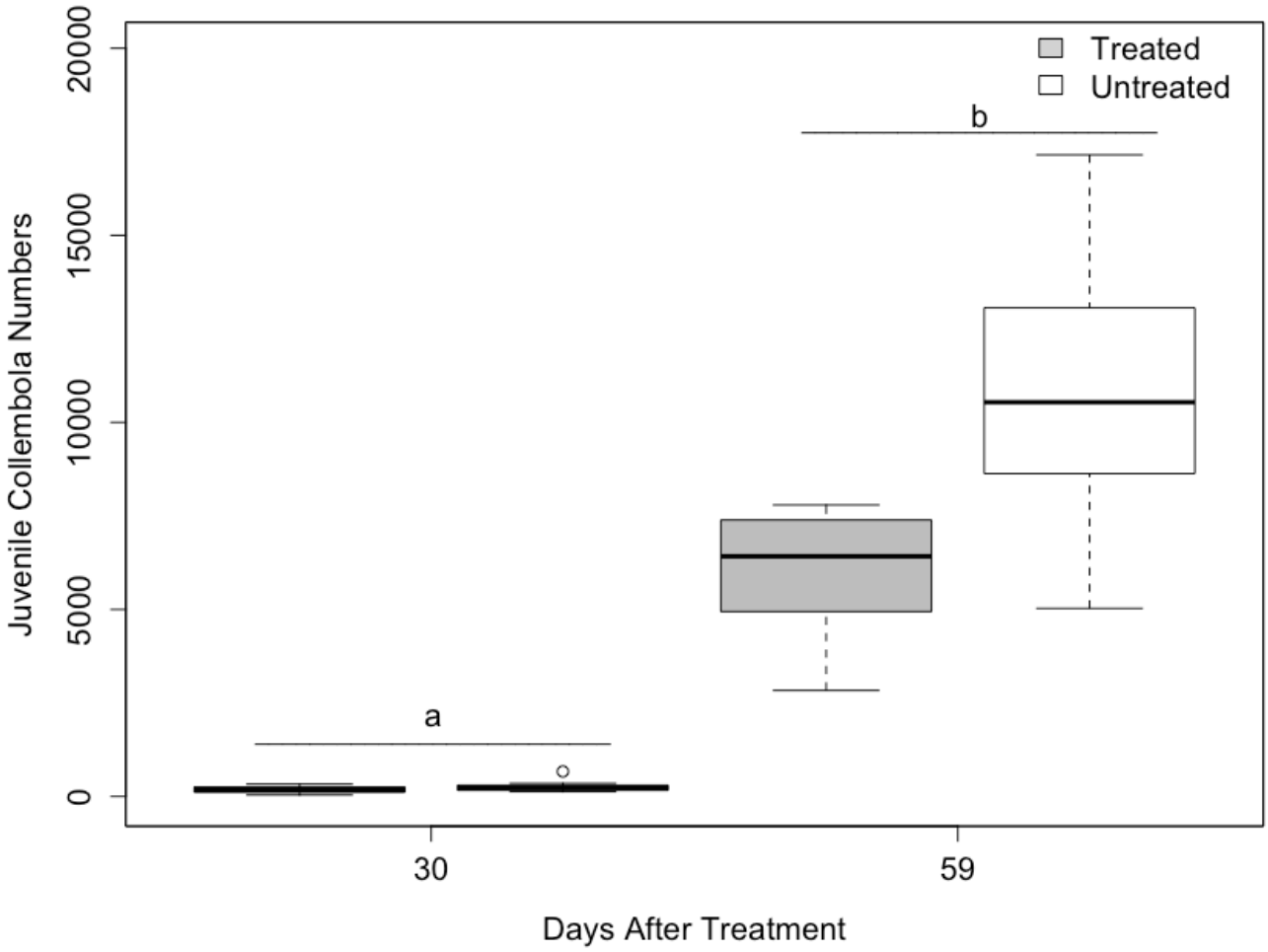


Figure 5.8. The juvenile Collembola (*F. candida*) numbers observed within litterbags in laboratory microcosms up to 126 days following a laboratory application of Garlon XRT (triclopyr). Bars represent means with standard error and different letters denote significant difference over time (ANOVA, TukeyHSD<0.05).

Collembola (*F. candida*) and mites (*O. nitens*) avoided leaf litter (*P. tremulodies, B. papyrifera*, and *S. bebbiana*) treated with Garlon XRT (triclopyr), but at relatively high concentrations (i.e., EC_{50} values were above the maximum application rate). The EC_{25} for Collembola avoidance was 1309 g a.i. ha^{-}, the EC_{50} was 5167 g a.i. ha^{-1}, and the EC_{70} was 19,269 g a.i. ha^{-1} (Figure 5.9). The EC_{25} for mite avoidance was 4084 g a.i. ha^{-1}, the EC_{50} was 23,597 g a.i. ha^{-1}, and the EC_{70} was 126,770 g a.i. ha^{-1} (Figure 5.10). For both Collembola and mites, the EC_{25} values fell below the maximum field application rate of 4530 g a.i. ha^{-1}, and the EC_{50} and EC_{70} values were above. Collembola were considerably more sensitive to triclopyr with an

avoidance EC_{25} 3.5 times below maximum field application compared to mites that were 1.1 times below. This is especially apparent when comparing EC_{50} values, as the avoidance response for Collembola was 1.1 times above maximum field application and the EC_{50} for mites was five times higher.

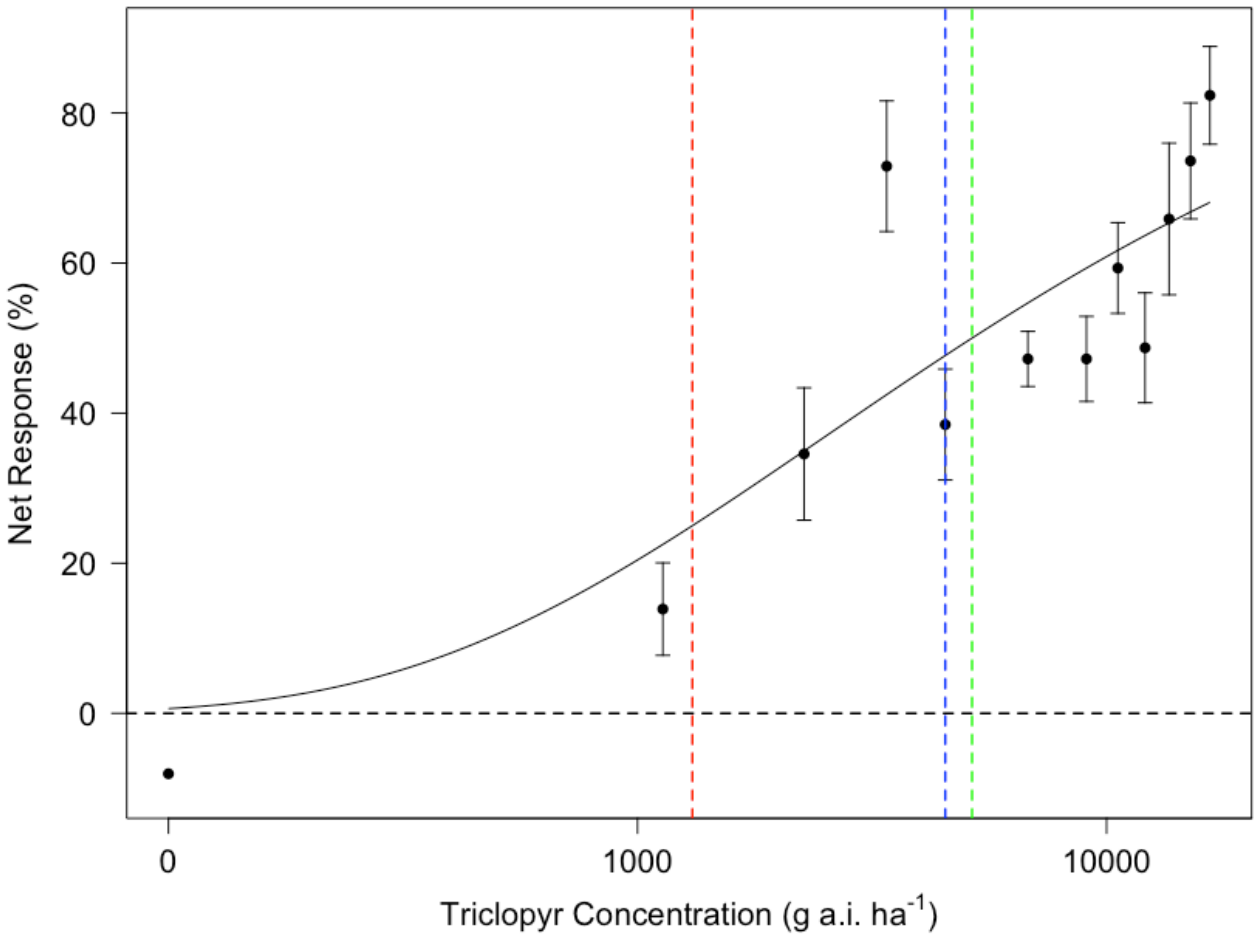


Figure 5.9. Collembola (*F. candida)* avoidance results from Garlon XRT (triclopyr). Net response expressed as a percentage (number of organisms on untreated side subtracted from number of organisms on treated side divided by total organism recovery) observed over different concentrations of Garlon XRT (triclopyr, g ai. ha^{-1}). Above black dotted line at zero is considered avoidance of and below zero is considered attraction to Garlon XRT (triclopyr). Red dotted line notes EC_{25} of 1309 g a.i. ha^{-1}. Blue dotted line represents maximum herbicide application rate of 4530 g a.i. $ha^{-1.}$ Green dotted line signifies EC_{50} of 5167 g a.i. ha^{-1}. The EC_{70} exceeds the concentration axis at 19,269 g a.i. ha^{-1}.

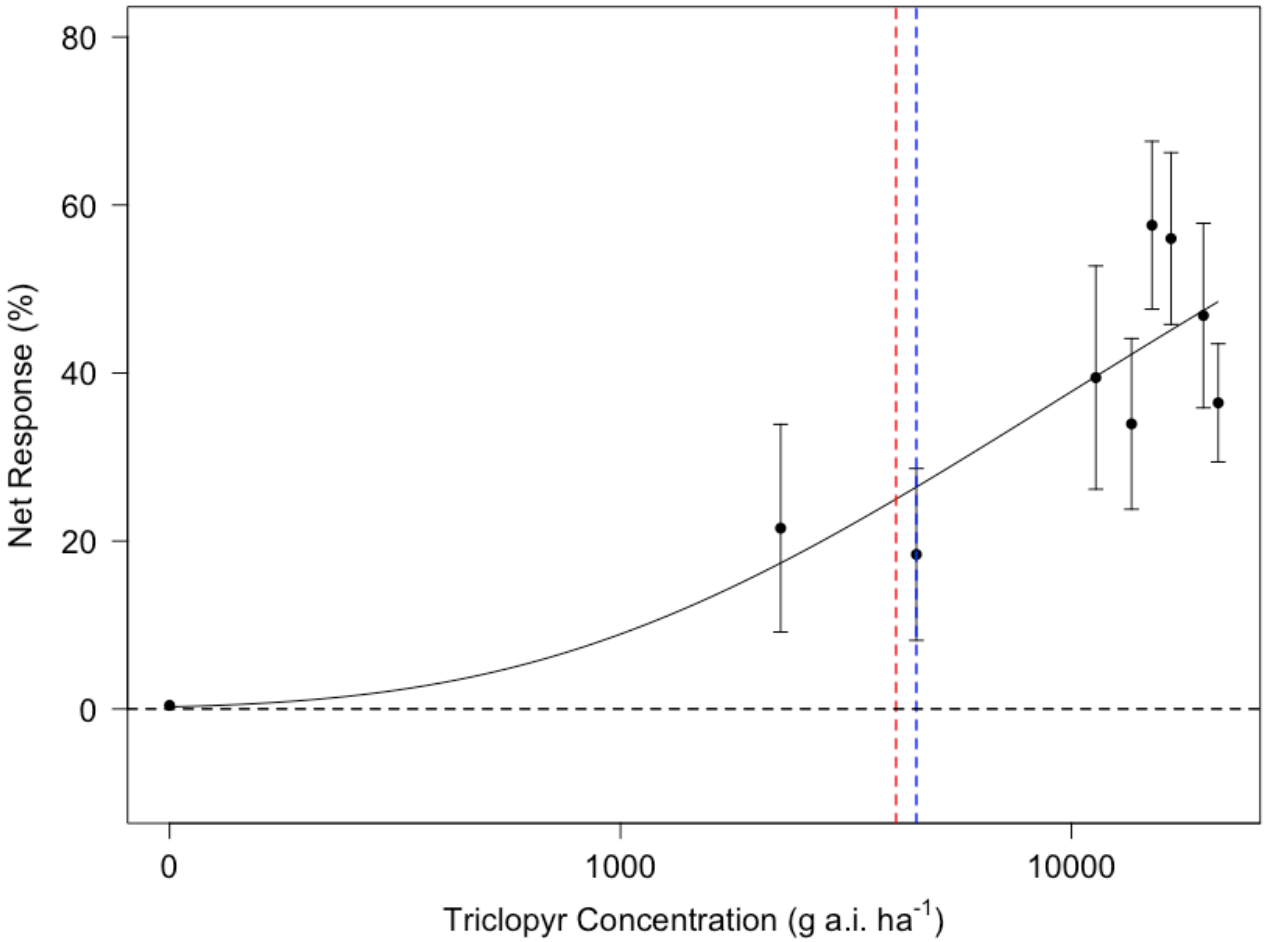


Figure 5.10. Mite (*O. nitens)* avoidance results from Garlon XRT (triclopyr). Net response expressed as a percentage (number of organisms on untreated side subtracted from number of organisms on treated side divided by total organism recovery) observed over different concentrations of Garlon XRT (triclopyr, g ai. ha^{-1}). Above black dotted line at zero is considered avoidance of and below zero is considered attraction to Garlon XRT (triclopyr). Red dotted line represents EC_{25} 4084 g a.i. ha^{-1}. Blue dotted line denotes maximum herbicide application rate of 4530 g a.i. ha^{-1}. The EC_{50} and EC_{70} exceeds the concentration axis at 23,597 g a.i. ha^{-1} and 126,770 g a.i. ha^{-1}, respectively.

5.5. Discussion

Garlon XRT (triclopyr) did not significantly reduce the breakdown of field treated *S. bebbiana* foliage and laboratory treated boreal foliage (*S. bebbiana*, *P. tremuloides*, and *B. papyrifera*) up to one year following treatment. In both field and microcosms studies there was a

negligible difference in mass loss between treated and untreated litter. Although litter quality measured via C:N ratio was found to differ in field litter where natural senescence of leaves following herbicide application occurred, the lower C:N ratio observed for treated litter did not appear to alter breakdown rates. Boreal invertebrates, key organisms in litter breakdown, had higher adult and juvenile abundance (*F. candida*) in untreated litter; however, there was no statistically significant difference between untreated and treated litter. An avoidance response was observed for both *F. candida* (EC_{50} = 5167 g ha^{-1}) and *O. nitens* (EC_{50} = 23,597 g ha^{-1}), but values were above the maximum application rate (4530 g ha^{-1}).

5.5.1. *Mass Loss and Quality of Litter*

Throughout the duration of our experiments there were negligible differences between mass loss of treated and untreated litter. To our knowledge there have been no studies that examine triclopyr and boreal leaf litter breakdown in terrestrial systems. However, triclopyr is a chlorophenoxyacetic acid herbicide, and the effects of herbicides on mass loss have been observed with other structurally similar chlorophenoxyacetic acid herbicides like 2,4,5-tricholorophenoxyacetic acid (2,4,5-T), 2,4-dicholorophenoxyacetic acid (2,4-D). Fletcher & Freedman (1986) found mass loss was only inhibited in treated litter once field application rates of these herbicides were exceeded fifty times. We observed mass loss in field *S. bebbiana* litter between 24% (untreated) and 28% (treated) a year following application. Similarly, there was no difference in mass loss in untreated and treated (2,4,5-T) white oak (*Quercus alba*) foliage (average 35%), stem injected in the field prior to senescence and collected after natural abscission (Gottschalk & Shure 1979). We observed mass loss between 46% (treated) and 47% (untreated) in laboratory treated mixed boreal foliage 126 DAT. This was comparable to a microcosm study that also collected litter prior to senescence, treated in a laboratory, and measured mass loss on unburied litter. Mass loss in untreated (34%) and treated (2,4-D and 2,4,5-T, 33 - 37%) red maple (*Acer rubrum*) foliage in microcosms was also not significantly different at 121 DAT (Fletcher & Freedman 1986). Litter decomposition can span several years and even decades, especially in cold dry regions like the boreal forest (Berg 2000; Parton et al. 2007). Few studies have invested in the time and resources required to effectively observe litter decomposition impacts on long-term ecosystem health. Longer-term studies are needed to determine if litter breakdown following application of triclopyr to boreal forest species is influenced at later stages (i.e., >365 days).

However, both our field and laboratory studies suggest that triclopyr application does not influence litter breakdown rates within the first year.

The rates of mass loss in our microcosm study (46 - 47% at 121 DAT) were much higher than our field study, likely influenced by the optimal conditions (60% soil water holding capacity, 20 ± 2 °C, 60% humidity, and 16-hour light and 8-hour dark photoperiod) maintained in the microcosms. The accumulation of organic matter from litter decomposition is a slow process, particularly in cold dry areas like the boreal forest (Berg 2000; Parton et al. 2007). In our field study, litterbags were buried in late fall and recovered following winter, when decay rates were retarded by cooler temperatures (Gottschalk & Shure 1979; Kim 2007). Within the first few months litter can lose up to 30% of its mass from the leaching of water-soluble compounds (Seastedt 1984; Bryant et al. 1998; Berg & McClaugherty 2003; Naiman 2005; Berg 2014). It is probable that most of the litter mass loss observed in our studies was the result of leaching soluble compounds stimulated in microcosms by consistent watering and temperature conditions. Finally, we used a mixture of boreal species (*S. bebbiana*, *P. tremuloides*, and *B. papyrifera*) in our microcosm study and only willow (*S. bebbiana*) in our field study, which may have influenced mass loss. In a litter species mixture, higher quality litter is preferentially decomposed. In litter mixtures there is greater nutrient availability for microbes, and nutrients can be redistributed to lower quality litter through fungal hyphal networks which improves local nutrient requirements and increases litter decomposition (Hattenschwiler et al. 2005; Gessner et al. 2010).

Higher nitrogen and consequently lower C:N profiles in field treated litter did not result in increased mass loss within the duration of our study. Herbicide application that occurs prior to natural leaf senescence causes nutrients that would have otherwise translocated from the leaves to stems and belowground structures to remain, resulting in increased nitrogen (lower C:N) and less recalcitrant lignin in treated foliage (Cromack & Monk 1975; Gottschalk & Shure 1979). Litter for the microcosm study was manually defoliated prior to senescence and treated with herbicide in the laboratory, which generated no difference in nitrogen concentration between treated and untreated litter. Whereas, *S. bebbiana* foliage used in field litterbags was sourced from willows treated in the field prior to senescence resulting in treated litter with nitrogen concentrations 2.5-fold higher than untreated. Nitrogen is a limiting resource for degrading microorganisms in boreal ecosystems and therefore litter with higher nitrogen can degrade at faster rates (Swift 1979; Blair et al. 1992;

Naiman et al. 2005; Kim 2007). However, no significant difference in mass loss was observed in either of our studies, even in the field trial with litter that had different C:N profiles. This is counter to what occurred in white oak (*Quercus alba*) treated by stem injection with 2,4,5-T (Gottschalk & Shure 1979). Treated white oak contained higher nitrogen in foliage and had breakdown rates that were as much as 1.5 times greater than untreated foliage. Similarly, more mass loss occurred at every time point up to a year in litter with higher nitrogen including three oak species (*Quercus mongolica*, *varibilis,* and *serrata*), yellow birch (*Betula allegheniensis*), sugar maple (*Acer saccharum*), and beech (*Fagus grandifolia*) (Gosz et al. 1973; Kim 2007). Some studies have reported initial C:N to be the best predictor of mass loss (Swift 1979; Tian et al. 1992; Kim 2007); however, others have suggested nitrogen rich litter inhibits later decomposition stages by reducing fungal activity involved in oxidizing plant polyphenols and lignin (Berg 2000; Zak et al. 2017).

In our laboratory microcosms C:N ratios decreased with time, but in our field study C:N ratios were only different between the initial litter and 280 DAT. However, overall there was a general decline in C:N ratios driven by increases in nitrogen in both microcosm and field studies. Typically, microorganisms accumulate exogenous nitrogen to satisfy their needs, as observed throughout the duration of our studies, until sufficient carbon is respired causing a decline in C:N to a critical breaking point at which time nitrogen mineralization occurs (Aber & Melillo 1982; McClaugherty et al. 1985; Manzoni et al. 2008; Agren et al. 2013). Generally, nitrogen mineralization occurs with C:N ratios between 20 to 40 (Cabrera et al. 2005; Kim 2007; Liu & Sun 2013). It is possible that treated litter with higher nitrogen (lower C:N) may undergo nitrogen mineralization at an accelerated rate compared to untreated litter (Bryant et al. 1998; Parton et al. 2007; Manzoni et al. 2008). Despite different C:N profiles between field treated (C:N decreased from 71 to 40) and untreated (C:N decreased from 29 to 19) foliage, the C:N decline over the duration of the field study was similar, decreasing between 1.8 (untreated) and 1.5 (treated) times the initial C:N values. Lower litter C:N did not influence litter breakdown rates and therefore habitat quality was not influenced by these parameters within the duration of our study.

5.5.2. *Effects to Boreal Invertebrates*

No difference in litter mass loss was observed between microcosms with or without inoculation of invertebrates. There was also no statistical difference in total *F. candida* or juvenile abundance between microcosms with herbicide treated and untreated litter. However, there was

consistently higher *F. candida* adult and juvenile abundance in untreated litter at every recovery period. This is counter to what was observed by Gottschalk & Shure (1979), who reported an increase in total Collembola and mite numbers for eight months following a chlorophenoxyacetic acid herbicide application, but was not enough to influence mass loss in litter. Sublethal effects from herbicides can induce a hormetic response, where there is an investment in survival of offspring through increased reproduction at the sacrifice of adult growth and individual survival (Van Straalen 1994; Puurtinen & Martikainen 1997). Alternatively, chlorophenoxyacetic acid herbicides can stimulate microbial populations that in turn stimulate microarthropod communities (Gottschalk & Shure 1979; Bolan & Baskaran 1996). There have been varying results on the effects of herbicide to litter degrading communities. Some studies on chlorophenoxyacetic acid herbicides have found no adverse effects on arthropod species richness or abundance, whereas others report the effect to be dependent on both invertebrate and leaf litter species (Bramble et al. 1999; Clark et al. 2009). Our microcosms study exhibited no significant difference in *F. candida* total abundance in treated and untreated litter or connection to litter mass loss differences between treatments.

Boreal invertebrates appear to be relatively insensitive to triclopyr. Jimmo (2018) determined *E. crypticus* to be the most sensitive boreal invertebrate to triclopyr with the lowest 28-day LC_{25} of five soil profiles being 220 ± 46 ug g soil^{-1}. This is above the concentration in field *S. bebbiana* foliage at the time of litter burial (177 ± 9 ug g dry biomass^{-1}). Interestingly, the dissipation rate constant of the buried litter was almost identical to that of the terminal dissipation phase (70 DAT to 365 DAT) in aboveground foliage (k = -0.49 for litter belowground and -0.48 aboveground). Suggesting that degradation of triclopyr at low concentrations, like those observed in our litter at the time of burial, may be consistent regardless of habitat location. Since microbial degradation is likely the primary degradation pathway at both locations, there may be similar degrading capacities for microbial populations on aboveground foliage and belowground litter. The concentration in treated foliage at the time of abscission did not appear to be high enough to influence soil invertebrates and/or litter breakdown. The concentration of triclopyr was much higher in the microcosm litterbags (2124 ± 84 ug g dry biomass^{-1} at time zero) and remained over *F. candida* LC_{25} values for the duration of the experiment (126 DAT). Total *F. candida* abundances in microcosms were not significantly different between treated and untreated litter despite triclopyr concentrations over LC_{25} reported by Jimmo (2018), possibly suggesting avoidance behaviour.

However, the relationship between triclopyr in a gram of soil and leaf litter is not a direct comparison and exposure in our microcosms may have been different from that in Jimmo (2018).

Avoidance tests are reflective of field conditions as there is opportunity for organisms to reduce exposure by evading harmful contaminants they detect and are generally a more sensitive endpoint compared to acute and chronic soil toxicity tests (Aldaya et al. 2006; Araújo et al. 2016). These tests are a relatively recent inclusion to toxicity testing and as such there is limited knowledge especially relating to boreal conditions and species (Racke 1993; Princz et al. 2012; van Gestel 2012). Five Collembolan species including *F. candida* displayed a significant avoidance response to the herbicide Betanal (a.i. phenmedipham), but were immobilized and unable to escape the contaminant when concentrations approached LC_{50} values (Heupel 2002). Boreal invertebrates in our study displayed an avoidance response to Garlon XRT (triclopyr), although impaired ecosystem services are unlikely to occur at field application rates. The EC_{25} avoidance for both *F. candida* (2068 g ha^{-1}) and *O. nitens* (4084 g ha^{-1}) were below triclopyr maximum application rate (4530 g ha^{-1}), while the EC_{50} values were above.

Boreal *O. nitens* were more tolerant to triclopyr than *F. candida* with an EC_{50} five times the application rate. Collembola have ventral tubes that aid in water absorption and waxy chitin cuticles that can leave them vulnerable to contaminants, especially those with water soluble characteristics (Gainer et al. 2018). Oribatid mites have hard sclerotized and chitinous exoskeletons that are less permeable further contributing to reduced susceptibility (Gainer et al. 2018). Jimmo et al. (2018) also demonstrated that *O. nitens* were orders of magnitude more tolerant to triclopyr than *F. candida*. Similarly, Collembola were found to be more sensitive to other pesticides like organophosphate insecticides and the herbicide imazapyr than mites (Jegede et al. 2017; Jimmo 2018). The EC_{25} for both *F. candida* and *O. nitens* were well above the LC_{25} and reproductive EC_{25} for these boreal species in soils (Jimmo et al. 2018), suggesting that avoidance is a more sensitive endpoint. We provide a very sensitive avoidance endpoint at EC_{25}; however, impairment of habitat services is usually only considered with an avoidance response greater than 70% (Hund-Rinke & Wiechering 2001; ISO 2011; Owojori et al. 2014). The EC_{70} values for both *F. candida* (4 times) and *O. nitens* (28 times) were well above maximum allowable application rates and therefore triclopyr application at field rates are unlikely to impair ecosystem services.

5.5.3. *Conclusion*

Herbicides can impact habitat quality by influencing litter decomposition rates, litter quality, and the important services soil invertebrates provide in nutrient cycling. However, our field and laboratory studies did not find triclopyr had a significant impact of ecosystem health or services measured via litter breakdown, litter quality, and invertebrate avoidance. Litter breakdown rates were not significantly different between treated and untreated litter up to a year following treatment, despite differences in C:N ratio of field treated litter. The EC_{25} avoidance response for both *F. candida* (2068 g a^{-1}) and *O. nitens* (4084 g ha^{-1}) were below triclopyr maximum application rate (4530 g ha^{-1}); however, the EC_{70} values where impairment of ecosystem services is expected were well above application rates for both invertebrates. The current maximum allowable application rates for Garlon XRT appear to be sufficiently protective for maintenance of terrestrial boreal habitat quality and boreal soil invertebrate species. Longer term studies examining litter decomposition rates beyond one year, as well as a closer examination of the long-term implications of treated litter with higher C:N ratios are needed. Our study provides novel information regarding the impacts of triclopyr on ecosystem health but is limited in scope. It is highly recommended that landscape scale application of triclopyr in boreal ecosystems is accompanied by further studies and monitoring to ensure the trends observed in this study apply on a larger scale.

6. SYNTHESES

6.1. PRINCIPAL FINDINGS

6.1.1. *Drift and Dissipation*

Triclopyr drift and dissipation in foliage was examined following two targeted herbicide field application techniques in boreal Saskatchewan - low-volume foliar and basal bark. The primary results from Chapter Four indicate that triclopyr drift concentrations from low-volume foliar and basal bark applications were generally below levels of ecological concern and concentrations dissipated relatively quickly (DT_{50} = 5.7 days and DT_{90} = 34.6 days) in foliage. Triclopyr drift concentrations following basal bark treatments were greater but concentrations were localized near the base of the stem. Concentrations in foliage were higher when herbicides were applied via low-volume foliar compared to basal bark application. The hazard quotient risk assessment extrapolated from interspecies toxicity endpoints indicates concentrations of triclopyr detected in this study are unlikely to illicit acute toxicity, but long-term browsing by wildlife on contaminated leaves could result in chronic effects, at least for moose.

6.1.2. *Habitat Quality*

Indirect effects of triclopyr on habitat quality were examined in Chapter Five through litter mass loss and quality (C:N ratios), as well as the response of boreal invertebrates (*Folsomia candida* and *Oppia nitens*) in microcosms and avoidance tests. Foliage treated with triclopyr in the field contained more nitrogen (lower C:N) than untreated foliage resulting from triclopyr repression of natural senescence processes. Literature suggests that litter with initially high nitrogen may decompose at a faster rate and nitrogen mineralization will occur earlier, possibly disrupting nitrogen cycling dynamics in soil systems. However, in our study despite nitrogen profile differences, litter breakdown rates were not significantly different within a year of treatment.

Adverse effects to boreal invertebrates and the ecological services they provide are improbable at field application concentrations. The low triclopyr concentration at the time treated leaves enter the soil ecosystem is also unlikely to cause adverse effects to soil invertebrates. The EC_{25} triclopyr avoidance values of *F. candida* and *O. nitens* were below field application rates and at field application concentrations there were no differences in survival and reproduction rates of

F. candida. Avoidance could influence ecosystem services at EC_{70} values, which were 4 and 28 times above maximum application rate for *F. candida* and *O. nitens*, respectively. Therefore, field application rates of triclopyr are unlikely to impair ecosystem services.

6.2. Industry Recommendations for Herbicide Application

6.2.1. *Application Technique*

Substantially less triclopyr was found in foliage following basal bark application compared with low-volume foliar treatments, and therefore basal bark is preferable over low-volume foliar application where chronic toxic effects for herbivores is of concern. There were greater localized drift concentrations surrounding the base of treated stems associated with basal bark treatments, but concentrations in our study were below the most sensitive toxicity endpoints. However, high stem density could exacerbate these effects and may elevate triclopyr input into the soil system. Low-volume foliar applications are best suited in areas with higher stem density when off-target herbicide deposition is less of a concern, and basal bark applications are ideal when stem density is lower and non-target deposition is a concern.

6.2.2. *Considerations of Boreal Landscapes and Soils*

Bogs and marshes account for approximately 4% and lakes for 18% of the I3P transmission right-of-way (ROW) in northern Saskatchewan (Beak Associates Consulting Ltd. 1989). Triclopyr can enter surface water through drift, runoff, or direct deposition from overspray. The ester form of triclopyr is toxic to fish and aquatic invertebrates, but often has a short half-life within two days, except in low pH and temperature conditions (Stewart 1991). Provincial and Federal law mandates a 25 m setback between ground herbicide application and water courses, which can cause challenges for integrated vegetation management (IVM) in northern Saskatchewan. The Ministry of the Environment has permitted the targeted low-volume basal bark and cut stump backpack treatments within 5 m of surface water in some situations. Triclopyr movement may be limited in areas with organic peat soil associated with bogs and fens; however, these regions also have poor drainage and can be more acidic which increases toxicity exposure (Beak Associates Consulting Ltd. 1989). Even at application rates there is unacceptable aquatic risk that could occur from overspray and surface water and therefore surveys of the treatment area should be conducted prior to application to mitigate any risks. Boreal soils typically have an acidic and poorly humified top layer high in organic matter (Thompson et al. 2000; Newton et al. 2008). This facilitates triclopyr

sorption to soil making triclopyr less available for microbial degradation which can decrease degradation rates and soil mobility (Stougaard et al. 1990; Johnson et al. 1995). The I3P transmission ROW is primarily comprised of sandy soil, shallow development of sandy/loamy soil over bedrock, and poorly drained organic peat soil in areas with bogs and fens (Beak Associates Consulting Ltd. 1989). Sandy soils can promote herbicide mobility and increase the probability of off-target effects. However, northern studies indicate that adverse environmental consequences are unlikely to occur from triclopyr persistence and mobility even in sandy soils (Jotcham et al. 1989; Stephenson et al. 1990; Stewart 1991; Pusino et al. 1994; Thompson et al. 2000; Newton et al. 2008). Caution should still be observed in areas with sandy soil to both minimize erosion and reduce soil exposure to herbicide. As discussed previously, basal bark applications deposited higher herbicide concentrations that were spatially limited around treated stems which can equate to elevated deposition with high stem density. The surrounding environment should be considered before treatment, especially in areas with sandy soil near slopes and surface water.

6.2.3. *Public Considerations*

There is more acceptance of herbicide use in southern Saskatchewan where pesticides are commonly used in agriculture. However, in northern regions where harvesting and use of native flora and fauna is more prevalent, herbicides are less accepted. There is still a perception of adverse risk associated with herbicide usage, regardless of scientific evidence that suggests risk from registered herbicides used according to label instructions is acceptable (Lautenschlager & Sullivan 2002). Social license for herbicide use is often more difficult to obtain because of different concerns pertaining to native flora and fauna, as well as economic situations. Few attempts have been made to capture the ecological and social concerns and knowledge of Indigenous communities in research and environmental decision-making, especially relating to alternative IVM approaches (Lautenschlager & Sullivan 2004; Stewart & Sinclair 2007; Brock 2019). Fair participation can be promoted by providing training and funding to support Indigenous communities in acquiring skills to make informed decisions and partaking in economic benefits that relate to Traditional Land use (Brock 2019). Furthermore, assisting with Indigenous directed Traditional Land use studies can provide understanding in how the land is being used when project development occurs and appropriate mitigation action can be taken when necessary. Frameworks should better address specific concerns Indigenous communities have on herbicide use (economic

and toxicity), and support alternative IVM treatments to facilitate more effective engagement (Lautenschlager & Sullivan 2004). Investing in collaborative decision-making and Indigenous communities in general, can facilitate knowledge exchange, build relationships, and improve engagement outcomes (Stewart & Sinclair 2007; Hall et al. 2015; Brock 2019).

6.3. Community Engagement

The impact of herbicides on northern transmission ROWs is especially concerning to many northern communities that have vested interests, and treaty and inherent rights associated with natural resources provided by the boreal forest. Hunting and gathering is prevalent in northern Saskatchewan. Not only are traditional foods culturally important to Indigenous communities, many are reliant on boreal resources for their livelihood. Moreover, tree removal has historically provided northern community members with employment and theoretically over time the use of herbicide will reduce the maintenance required to remove trees and employment opportunities for which community members are currently trained. Triclopyr and end-use products have been registered in Canada since 1991 for ground application in industry and woodland sites, and as such SaskPower has a legal license for use (Stewart 1991). However, local communities have expressed concern with SaskPower's proposed targeted use of Garlon RTU on northern ROWs on traditional territory.

Indigenous engagement in environmental decision-making on traditional territory can allow communities to participate in and adapt to changes that affect them (Stewart & Sinclair 2007; Brock 2019). Effective engagement from industry can provide benefits such as access to traditional knowledge, innovative and balanced resolutions for environmental concerns, and possible avoidance of taxing litigations when there is project opposition. However, the process is often expensive, time consuming, poorly executed, and ineffective. Moreover, in Canada, Indigenous people hold distinct rights and interests from public stakeholders and often have unique mandates and motivations (Brock 2019). This contributes to the ambiguous understanding of the roles and responsibilities involved in effective engagement practices that can cause a disconnect in outcome expectations leading to frustration and project resistance (Stewart & Sinclair 2007; Brock 2019). Effective engagement can also be complicated by the range in communication patterns and paradigms of knowledge (Stewart & Sinclair 2007).

A significant barrier to meaningful public and Indigenous participation is a clear understanding of the project and its consequences (Stewart & Sinclair 2007; Vousden et al. 2014). Often environmental decisions are complex and require comprehension of specific scientific knowledge on various spatial and temporal scales (Benda et al. 2002). Therefore, it is important that ongoing, adequate and accessible information is available to all participants. This process can take time and ideally involves numerous opportunities and techniques to promote engagement and understanding of the issues for people of varying backgrounds (Stewart & Sinclair 2007).

Professional knowledge is almost exclusively used in environmental decision-making despite intrinsic bias in how scientific knowledge is used to address environmental challenges (Wilkinson et al. 2007). Empirical data accounts for the physical effects and is necessary, but does not always sufficiently consider contextual knowledge gained from experience and often ignores spiritual connections and traditional perspectives (Wilkinson et al. 2007; Kayahara & Armstrong 2015). Scientific evidence suggests concerns regarding adverse effects of registered herbicides used according to label instructions are unwarranted (Lautenschlager & Sullivan 2002). However, there are numerous examples of herbicides regarded as safe for use by regulatory frameworks that were later discovered to be harmful to humans, wildlife, and/or the ecosystem like dichlorodiphenyltrichloroethane (DDT), 2,4,5-tricholorophenoxyacetic acid (2,4,5-T), and neonicotinoids (Kayahara & Armstrong 2015). Furthermore, the negative legacy of colonialism has provided several reasons for Indigenous communities to distrust regulatory frameworks from governing agencies. These challenges make it difficult to communicate, understand, and relate to messages and knowledge across varying backgrounds and perspectives. More productive solutions may come from engagement efforts that work to develop relationships, trust, and understanding before assertive educational attempts used to justify the use of herbicides considered to have minimal associated risks (Kayahara & Armstrong 2015).

Working in partnership with SaskPower and the Lac La Ronge Indian Band, this project was designed to address some of the herbicide toxicity concerns of local communities. Initially there were open discussions with the Lac La Ronge Indian Band and SaskPower prior to project objectives to understand the context of the concerns, provide information, and respond to questions. This project included two four-month internships with SaskPower where I assisted with activities that improved the comprehension of IVM to effectively communicate the risks and

benefits to external stakeholders. During these internships, research plots were established and monitored for herbicide efficacy and vegetation community differences between herbicide treated and untreated portions of a ROW to provide insight into local long-term IVM effects. Ongoing updates on overall project progress, results, and concerns were addressed with the Lac La Ronge Indian Band, the Northern Saskatchewan Environmental Quality Committee, and the Lands and Resources Management Board over a period of four years. Members of the Lac La Ronge Indian Band, SaskPower, and the Ministry of Environment participated in a final workshop which included informational posters in English and Cree and an open discussion on any additional concerns. The posters, "How can herbicides affect wildlife and the environment?" and "Herbicides and ecosystem health," were developed to communicate the research in this project and I facilitated the discussion on herbicides and ecosystem health (Appendix F). Throughout the project our team realized the importance and embraced several engagement opportunities with northern communities.

Our team engaged northern communities in several ways to encourage a holistic understanding of IVM. This included teaching classes at various elementary and high schools in northern Saskatchewan, collaborating with the Keewatin Community Development Association (KCDA) on projects, and building an informative IVM website. Classes engaged youth in plant surveying skills taught alongside a local elder to facilitate traditional and scientific knowledge sharing, vegetation manipulation to improve understanding of IVM concepts and practices, and the purpose and procedures of duty to consult and accommodate. I participated in the youth education programs by teaching and overseeing activities related to plant species identification, plant surveying techniques, and the use and necessity of different IVM tools. A native seed collection pilot project with SaskPower and the KCDA was also initiated to address remaining questions regarding feasible IVM options for boreal ROWs and examine the viability of incorporating wild products harvesting. This included the community-based collection of native seeds, greenhouse germination, and the planting and monitoring of boreal plugs. I assisted in planting boreal plugs and monitoring the growth and survival the following month. Additionally, a website (https://ivm-row.wixsite.com/ivm-row) was created to provide accessible knowledge on IVM in boreal Saskatchewan and includes information on IVM and herbicide use, engagement, and duty to consult and accommodate, and specific project details. Investing in community

engagement and participation is a critical aspect in gaining social licenses and effective IVM on ROWs.

6.4. Future Direction

The overall risk of targeted triclopyr use in northern Saskatchewan ROWs appears low based on the evidence provided from our field and laboratory experiments and the information available in the current literature. However, knowledge regarding herbicide use in northern regions is less robust than in southern regions and as a result there are some uncertainties surrounding adverse effects. Furthermore, to improve our ability to effectively communicate and discuss risks associated with herbicides new engagement strategies are needed.

It is evident that more research is necessary to address specific concerns regarding plant and animal species in boreal ecosystems. This is especially important for herbicide use near northern communities where hunting and gathering is prevalent and boreal plants and animals may be relied on for cultural purposes and livelihood. For instance, browsing habits of species that are of cultural importance, like moose and hares, are not well defined on ROWs following herbicide treatments and may be very dependent on local ecosystems. Additionally, litter decomposition provides critical nutrients to soil ecosystems and there is a need for longer-term studies that address indirect herbicide effects on treated leaf litter decomposition in boreal ecosystems. There is also minimal information on the effects of basal bark and cut stump treatments. With both methods, herbicide is translocated into woody tissue and roots, which was not addressed in this study. More research on basal bark and cut stump applications that focus on the translocation of herbicide to roots and the resulting dissipation dynamics are required to better understand herbicide efficacy and ecosystem effects. Alongside the need for improved toxicological and ecological data concerning herbicide use on boreal ROWs is a need for improved engagement processes to support risk communication and collaborative IVM decision-making.

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APPENDIX A: ECOLOGICAL SITE CHARACTERISTICS

TABLE LIST

Table A.1. Summary of site information and terminal ends of the I3P transmission right-of-way including GPS co-ordinates from the southern margin of the sites, ecoregion, treatment application date, mean annual precipitation (ppt), and mean January (Jan.) and July temperatures (temp.).

SaskPower proposed the use of Garlon RTU (triclopyr) to manage ROW vegetation on the I3P transmission line which overlays Treaty 10 Territory. The Lac La Ronge Indian Band has the largest overlapping territory with the I3P transmission line and did not approve the ROW as a test site for herbicide application. Drift and dissipation following basal bark treatment of aspens (*Populus tremuloides*) saplings and low-volume foliar treatment of willows (*Salix bebbiana*) shrubs were examined at two sites (Aspen and Willow). Both sites were located on the PA8 transmission right-of-way (ROW) along Highway 55 between Debden and Big River, Saskatchewan (Section 4.2.1, Figure 4.1). The PA8 transmission line is one of the most northern transmission lines where herbicide is currently applied, and it was selected to best emulate the vegetation and environmental conditions of more northern ROWs within Saskatchewan (Table A.1). Herbicide applications were applied by licensed applicators of Davey Tree Company of Canada Ltd., contracted by SaskPower.

Table A.1. Summary of site information and terminal ends of the I3P transmission right-of-way including GPS co-ordinates from the southern margin of the sites, ecoregion, treatment application date, mean annual precipitation (ppt), and mean January (Jan.) and July temperatures (temp.).

Site	Coordinates	Ecoregion	Mean Annual ppt† (mm)	Mean Jan. Temp† (°C)	Mean July Temp† (°C)
Aspen	53.61° N, 106.95° W	Boreal Transition	454.5	-16.9	16.5
Willow	53.66° N, 106.99° W				
La Ronge	55.21° N, 105.32° W	Churchill River Upland	486.2	-18.8	17.5
Island Falls	55.53°N 102.36° W		486.5	-22.2	17.3
Points North Landing	58.25° N, 104.12° W	Athabasca Plain	497.5	-23.6	15.8

†Weather data obtained from Environment Canada 1981-2010 Climate Normals: Medstead, closest station to the Aspen and Willow sites; La Ronge A Station, closest weather station to the site north of La Ronge; Island Falls Station, closest weather station to Island Falls; Cigar Lake Station, closest weather station to Points North Landing (Environment Canada 2017).

Materials for laboratory studies were collected within Lac La Ronge Indian Band Traditional Territory from a site with similar ecological characteristics to that of the I3P transmission line to ensure local relevance of the study. The majority of the I3P ROW is within the Churchill River Upland Ecoregion; however, the most northern portion of the line near Wollaston Lake, Saskatchewan is within the Athabasca Plain Ecoregion. Past glaciation within the Churchill River Upland Ecoregion has resulted in a rolling topography with bedrock outcrops, glacial deposits, and numerous lakes and wetlands (Secoy 2006; UofS 2008). Aspen and paper birch are associated with the Grey Luvisols on clayey lacustrine uplands and are diagnostic species for the Boreal Shield Ecozone, which encompasses the Churchill River Upland Ecoregion. Willow species are common diagnostic species for Wetland Ecosites in the area (McLaughlan et al. 2010; SKCDC 2014).

Leaves from paper birch *(Betula papyrifera*), aspen (*Populus tremuloides*), and willow (*Salix bebbiana*), as well as soil samples were collected from a site along Highway 102 located approximately 10 km north of Lac La Ronge, Saskatchewan (La Ronge site). The La Ronge site was selected because it resides within the Churchill River Upland Ecoregion and therefore shares many ecological characteristics (ie. soil type and vegetation species) with that of the I3P transmission ROW (Table A.1.) (McLaughlan et al. 2010).

References:

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APPENDIX B: TRICLOPYR ANALYSIS AND METHOD VALIDATION

Herbicide residue analysis of glass microfiber filter papers from the drift study, *P. tremuloides* leaves, and *S. bebbiana* leaves were conducted using modified procedures provided by Dow AgroSciences Canada Inc, Calgary, A.B (Tessier 2013; Lynn & Slinkard 2014).

LIST OF TABLES

Table B.13. Intra-day accuracy and precision for validation of triclopyr concentration in vegetation.

Table B.14. Inter-day accuracy and precision for validation of triclopyr concentration in vegetation.

Table B.15. Method validation results for sensitivity, carry-over, extraction recovery, and the matrix factor.

LIST OF FIGURES

B.1. Materials and Apparatus

B.1.1. Analytical Standard and Internal Standard

Stable isotope triclopyr [M+4] (3,5,6-trichloro(4,5,6-$^{13}C_3$,^{15}N)pyridin-2-yl)oxy)acetic acid), Dow AgroSciences Canada Inc.

TCP (3,5,6-trichloro-2-pyridinol), $C_5H_2Cl_3NO$, Toronto Research Chemicals

Triclopyr (3,5,6-trichloro-2-pyridinyl)oxy)acetic acid), Toronto Research Chemicals

B.1.2. Reagents

Acetic acid (glacial, ACS plus grade), Fisher Scientific

Acetonitrile (Chromasolv HPLC grade), Sigma Aldrich

Acetone (Chromasolv HPLC grade), Sigma Aldrich

Ammonium Acetate (ACS plus grade), Fisher Scientific

Formic acid (98%), EDM Chemicals

Glycerol (ACS plus grade), Fisher Scientific

Hydrochloric acid (12.1 M), Fisher Scientific

Methanol (Chromasolv HPLC grade), Sigma Aldrich

Milli-Q Ultrapure Water generated by Milli-Q A10 purification system

Sodium hydroxide (ACS plus grade), Fisher Scientific

B.1.3. Laboratory Equipment

AB Sciex 4000 hybrid triple quadrupole linear ion trap mass spectrometry, AB Sciex (Concord, ON, Canada)

Agilent 1260 Infinity II High Performance Liquid Chromatography, Agilent Technologies (Santa Clara, CA, USA)

Analytical balance (readability to 0.0001 g), Mettler Toledo AL204

Ball-grinder, Retsch MM-400

Centrifuge, Thermo ST16 with a TX-400 bucket

Combitips, various sizes between 0.010 – 50 mL capacity, Eppendorf

Flatbed shaker, 180 revolutions per minute, custom build

InfinityLab Poroshell 120 EC-C18 (4.6 × 50 mm, 2.7 µm), Agilent (Cat #699975-902)

Oasis HLB 3cc (60 mg), Waters

Pipette and tips (0.0005 mL – 2 mL), Eppendorf Research Plus or Eppendorf Reference 2

Polypropylene Falcon conical centrifuge tubes (15 mL and 50 mL capacity), Fisher Scientific

Repeater E3 Pipet, Eppendorf

Solid phase extraction manifold, Waters

Standard laboratory glassware (volumetric flasks, measuring cylinders)

Strata-X 33um (30 mg), Phenomenex

Strata-XL-A 100um (30 mg), Phenomenex

TurboVap nitrogen evaporator, Labconco (Kansas City, MO)

Ultrasonic bath, VWR B2500A-MT

Vortex, American Scientific Products

B.2. Drift Sample Preparation

Preparation of standards, quality controls, and samples for solid phase extraction (Table B.1 and Table B.2):

a. Prepare 0.6 ug mL^{-1} triclopyr in acetone Stock 1 solution for eight standard concentrations in drift analysis. Keep covered with parafilm when not in use to avoid evaporation.

b. Prepare 5 ug mL^{-1} triclopyr in acetone Stock 1 solution for three quality control concentrations in drift analysis. Keep covered with parafilm when not in use to avoid evaporation.

c. Aliquot the Stock 1 Volume from the Stock 1 Concentration into 15 mL Falcon tubes as described in Table B.1 and Table B.2. Add acetone to a final volume of 5 ml to equal the Stock 2 Concentration. Keep lid on when not in use to avoid evaporation.

d. Prepare 0.27 ug mL^{-1} triclopyr internal standard in acetone Stock 2 Concentration to add to samples, standards, quality controls, and a matrix blank. Keep covered with parafilm when not in use to avoid evaporation.

e. Aliquot the Stock 2 Volume from the Stock 2 Concentration into 15 mL Falcon tubes as described in Table B.1 for Garlon XRT and Table B.2 for Garlon RTU samples. Keep lid on when not in use to avoid evaporation.

f. Add 100 µL of an 80:20 methanol:glycerol solution and 100 uL 0.270 ug mL^{-1} triclopyr internal standard to all samples, standards, quality controls, and a matrix blank. Add 100 µL of an 80:20 methanol:glycerol solution to another matrix blank (no internal standard). Bring volume to 5 mL for Garlon XRT samples or 2 mL for Garlon RTU samples with acetone.

g. Vortex to mix for 30 seconds and centrifuge the samples for 5 minutes at 3500 rpm.

h. Evaporate the extract solution using a TurboVap nitrogen evaporator set at 40 °C with nitrogen flow rate of approximately 15 psi.

i. Add 0.5 mL methanol (10% of reconstitution fluid as extraction solvent:90% water) to samples. Vortex to mix for 1 minute, followed by 30 minutes of sonication in warm water bath. Add 4.5 mL of Milli-Q water to each sample and vortex.

j. Centrifuge samples for 5 minutes at 3500 rpm. The final sample solutions were purified using solid phase extraction (SPE). Drift samples from Garlon XRT were purified via polymeric reversed-phase sorbent using separate Waters Oasis HLB (60 mg/3 mL) SPE cartridges described in Step B.3.1. Drift samples from Garlon RTU were purified via polymeric strong anion sorbent using separate Phenomenex Strata XL-A (30 mg/1mL) SPE cartridges as described in Step B.3.2.

Table B.1. Preparation of standards, quality controls, and samples for solid phase extraction for Garlon XRT triclopyr drift analysis.

St. and QC	Stock 1 Conc. (ug/mL)	Stock 1 Vol. (mL)	Final Vol. (mL)	Stock 2 Conc. (ug/mL)	Stock 2 Vol. (mL)	Evap. Vol.* (mL)	Evap. Conc.* (ug/mL)	Final Vol. (mL)	Final Conc. (ug/mL)
St. 1	0.6	0.040	5	0.005	1.250	5	0.001	1	0.006
St. 2	0.6	0.080	5	0.010	1.250	5	0.002	1	0.012
St. 3	0.6	0.160	5	0.019	1.250	5	0.005	1	0.024
St. 4	0.6	0.200	5	0.024	1.250	5	0.006	1	0.030
St. 5	0.6	0.280	5	0.034	1.250	5	0.008	1	0.042
St. 6	0.6	0.320	5	0.039	1.250	5	0.010	1	0.048
St. 7	0.6	0.360	5	0.043	1.250	5	0.011	1	0.054
St. 8	0.6	0.400	5	0.048	1.250	5	0.012	1	0.060
LQC	5	0.180	5	0.180	0.100	5	0.004	1	0.018
MQC	5	0.360	5	0.360	0.100	5	0.007	1	0.036
HQC	5	0.510	5	0.510	0.100	5	0.010	1	0.051
Sample				0.03-14.00†		5		1	
IS‡				0.270	0.100	5	0.0054	1	0.027

* Evaporation volume of acetone depends on volume of field samples being analyzed at the time and ranged from 2 - 10 mL, therefore also affecting the concentration before nitrogen evaporation. However, most standards, quality controls, and samples were evaporated with a volume of 5 mL.

† Volume of field sample analyzed ranged between 0.03 – 14.00 mL.

‡ Internal standard was added to all standards, quality controls, samples, and matrix blank before nitrogen evaporation.

Table B.2. Preparation of standards, quality controls, and samples for solid phase extraction for Garlon RTU triclopyr drift analysis.

St. and QC	Stock 1 Conc. (ug/mL)	Stock 1 Vol. (mL)	Final Vol. (mL)	Stock 2 Conc. (ug/mL)	Stock 2 Vol. (mL)	Evap. Vol.* (mL)	Evap. Conc.* (ug/mL)	Final Vol. (mL)	Final Conc. (ug/mL)
St. 1	0.6	0.050	5	0.006	1.000	2	0.003	1	0.006
St. 2	0.6	0.100	5	0.012	1.000	2	0.006	1	0.012
St. 3	0.6	0.200	5	0.024	1.000	2	0.012	1	0.024
St. 4	0.6	0.250	5	0.03	1.000	2	0.015	1	0.030
St. 5	0.6	0.350	5	0.042	1.000	2	0.021	1	0.042
St. 6	0.6	0.400	5	0.048	1.000	2	0.024	1	0.048
St. 7	0.6	0.450	5	0.054	1.000	2	0.027	1	0.054
St. 8	0.6	0.500	5	0.06	1.000	2	0.03	1	0.060
LQC	5	0.180	5	0.180	0.100	2	0.009	1	0.018
MQC	5	0.360	5	0.360	0.100	2	0.018	1	0.036
HQC	5	0.510	5	0.510	0.100	2	0.0255	1	0.051
Sample				0.005-10.00†		2		1	
IS‡				0.270	0.100	2	0.0135	1	0.027

* Evaporation volume of acetone depended on volume of field samples being analyzed at the time and ranged from 2 - 10 mL, therefore also affecting the concentration before nitrogen evaporation. However, most standards, quality controls, and samples were evaporated with a volume of 2 mL.

† Volume of field sample analyzed ranged between 0.005 – 10.00 mL.

‡ Internal standard was added to all standards, quality controls, samples, and matrix blank before nitrogen evaporation.

B.3. Drift Sample Purification

B.3.1. Garlon XRT

Garlon XRT drift samples, standards, quality controls, and matrix blanks were purified via polymeric reversed-phase sorbent using separate Waters Oasis HLB (60 mg/3 mL) SPE cartridges by the following procedure (Table B.3):

a. Place separate Waters Oasis HLB (60 mg/3 mL) SPE cartridge on the vacuum manifold for each sample.

b. Condition SPE cartridge with 3.0 mL of methanol followed by 3.0 mL of Milli-Q water to equilibrate. Dry the SPE cartridge under full vacuum for 5 seconds between solvents.

c. Load samples from Step B.2. - j on to SPE cartridges. Draw samples through cartridges at a flow rate of approximately 0.5 mL min^{-1} and discarding eluate.

d. Rinse sample Falcon tube with 2 × 3 mL of Milli-Q water and load on to SPE cartridge at a flow rate of approximately 10 mL min^{-1}. Dry cartridge for 60 seconds under full vacuum.

e. Elute triclopyr from SPE cartridges with 2 × 1.0 mL aliquots of methanol and collect in 15 mL Falcon tubes. Dry cartridges under full vacuum for 30 seconds after each aliquot.

f. Add 50 µL of an 80:20 methanol:glycerol solution to each sample. Vortex for 30 seconds. Centrifuge the samples for 5 minutes at 3500 rpm.

k. Evaporate the eluate to complete dryness using a TurboVap nitrogen evaporator set at 40 °C with nitrogen flow rate of approximately 15 psi.

g. Add 0.5 mL methanol to samples. Vortex the samples for 1 minute, followed by 10 minutes of sonication in warm water bath.

h. Add 0.5 mL of Milli-Q water to each sample and vortex. Centrifuge the samples for 5 minutes at 3500 rpm

i. Load the purified samples into 2 mL glass autosampler vials and cap. Samples analyzed via liquid chromatography tandem mass spectrometry (LCMS/MS) with the lowest level of quantification equating to 0.020 g triclopyr ha^{-1} (Equation B.1).

Equation B.1

$$\text{triclopyr in drift samples (g ha}^{-1}\text{)} = \frac{\frac{\text{extraction conc.(ug mL}^{-1}\text{)}}{Vs} \times \text{Ve}}{\text{area } (cm^2)} \times \frac{1\ g}{1e^6\ ug} \times \frac{1e^8\ cm^2}{1\ ha}$$

where the extraction concentration is the concentration of standard 1 (0.006 ug mL^{-1}), Vs is the volume of the sample used (14 mL, for Garlon XRT), Ve is the volume of the entire sample

collected on the petri dish (30 mL), and area refers to the area of the petri dish sample was collected on (63.6 cm^2).

Table B.3. Solid phase extraction (SPE) procedure for Garlon XRT and Garlon RTU drift and vegetation (veg.) samples.

Samples	Garlon XRT Drift	Garlon RTU Drift	Garlon XRT and RTU in veg.
Extraction Cartridge	Waters Oasis HLB 3cc (60 mg)	Phenomenex Strata-XL-A 100um (30 mg)	Phenomenex Strata-X 33um (30 mg)
Extraction Method	polymeric reversed-phase sorbent	polymeric strong anion sorbent	polymeric reversed-phase sorbent
SPE Primer	methanol, then Milli-Q water	methanol, then Milli-Q water	acetonitrile, then 1.0 N hydrochloric acid
SPE Rinse	Milli-Q water	25 mM ammonium acetate, then methanol	acetonitrile/Milli-Q water/1N hydrochloric acid (30/69/1, v/v/v)
SPE Elude	methanol	methanol with 5% formic acid	acetonitrile/Milli-Q water/1N hydrochloric acid (60/39/1, v/v/v)

B.3.2. Garlon RTU

Samples from Garlon RTU, standards, quality controls, and matrix blanks were purified via polymeric strong anion sorbent using separate Phenomenex Strata XL-A (30 mg/1 mL) SPE cartridges by the following procedure (Table B.3):

a. Place separate Phenomenex Strata XL-A (30 mg/1 mL) SPE cartridges on the vacuum manifold for each sample.

b. Condition SPE cartridge with 1.0 mL of methanol followed by 1.0 mL of Milli-Q water to equilibrate. Do not let cartridge dry between solvents.

c. Load samples from Step B.2. - j on to SPE cartridges. Draw samples through the cartridges at a flow rate of approximately 0.5 mL min^{-1} and discard eluate.

d. Rinse sample Falcon tube with 1 mL 25mM ammonium acetate and load onto the SPE cartridge. Dry cartridge under full vacuum and discard eluate.

e. Soak the cartridge with 1 mL methanol for 2 min, dry under full vacuum, and discard the eluate. Less than 1% of the triclopyr is lost in this eluate.

f. Elute triclopyr from SPE cartridge with 2 × 0.500 mL aliquots of 5% formic acid in methanol and collect in 15 mL Falcon tubes. Dry cartridge under full vacuum for 30 seconds after each aliquot.

g. Add 50 µL of 80:20 methanol:glycerol solution to each sample. Vortex to mix for 30 seconds and centrifuge the samples for 5 minutes at 3500 rpm.

h. Evaporate eluate to complete dryness using a TurboVap nitrogen evaporator set at 40 °C with nitrogen flow rate of approximately 15 psi.

i. Add 0.5 mL methanol to samples. Vortex the samples for 1 minute, followed by 10 minutes of sonication in warm water bath.

j. Add 0.5 mL of Milli-Q water to each sample and vortex. Centrifuge the samples for 5 minutes at 3500 rpm.

k. Load the purified samples into 2 mL glass autosampler vials and cap. Samples to be analyzed via LCMS/MS with the lowest level of quantification equating to 0.028 g triclopyr ha^{-1} (Equation B.1).

B.4. Vegetation Sample Preparation

Preparation of vegetation standards, quality controls, and samples for solid phase extraction (Table B.4 and Table B.5):

a. Freeze-dry leaves (stored at -20 °C) and homogenize with a ball-grinder for use in triclopyr analysis.

b. Weigh 0.50 g sample into 15 mL Falcon tubes for dissipation in field willow (*Salix bebbiana*), aspen (*Populus tremuloides*), and litterbag samples or 0.25 g for microcosm and avoidance samples.

c. Prepare a 10, 25, and 200 ug mL^{-1} triclopyr in acetone Stock 1 solution for standard concentrations for vegetation analysis. Keep covered with parafilm when not in use to avoid evaporation.

d. Prepare a separate 200 ug mL^{-1} triclopyr in acetone Stock 1 solution for three quality control concentrations for vegetation analysis. Keep covered with parafilm when not in use to avoid evaporation.

e. Aliquot Stock 1 Volume from Stock 1 Concentration as described in Table B.4 or Table B.5 depending on vegetation sample weighed into 15 mL Falcon tubes and add acetone to a final volume of 10 mL to equal Stock 2 Concentration. Keep lid on when not in use to avoid evaporation.

f. Prepare an 8.5 ug mL^{-1} triclopyr internal standard in acetone Stock 2 Concentration to add to samples, standards, quality controls, and a matrix blank. Keep covered with parafilm when not in use to avoid evaporation.

g. Aliquot Stock 2 Volume from Stock 2 Concentration into 15 mL Falcon tubes as described in Table B.4 or Table B.5 depending on vegetation sample weight.

h. Add 100 uL or 50 uL (depending on vegetation sample weight) of the 8.5 ug mL^{-1} triclopyr internal standard to all samples, standards, quality controls, and one of two matrix blanks.

i. Bring volume to 10 mL or 5 mL with methanol/2.5 N sodium hydroxide (90/10, v/v) Extraction Volume depending on vegetation sample weight.

j. Vortex samples for 1 minute, shake on flat-bed shaker for 60 minutes at 180 excursions per minute, and centrifuge for 5 minutes at 3500 rpm.

k. Aliquot 0.2 mL sample extract into 0.8 mL methanol/2.5 N sodium hydroxide (90/10, v/v) Extraction Volume and 1.0 mL of the 1.0 N hydrochloric acid. Note that samples below the lowest level of quantification were later prepared using 1 mL of the sample Extract Volume (instead of 0.2 mL) and 1.0 mL of the 1.0 N hydrochloric acid.

l. Mix by vortex for one minute and centrifuge for 5 minutes at 3500 rpm. Samples from vegetation treated with Garlon XRT and Garlon RTU were purified via polymeric reversed-phase sorbent using Phenomenex Strata-X (30 mg/1 mL) SPE cartridges.

Table B.4. Preparation of standards, quality controls, and samples for solid phase extraction for field willow (*Salix bebbiana*), aspen (*Populus tremuloides*), and litterbag triclopyr vegetation analysis.

St. and QC*	Stock 1 Conc. (ug/mL)	Stock 1 Vol. (mL)	Final Vol. (mL)	Stock 2 Conc. (ug/mL)	Stock 2 Vol. (mL)	Extr. Vol. (mL)	Extr. Conc. (ug/mL)	SPE Vol. (mL)	Final Conc. (ug/mL)
St. 1	10	0.125	10	0.125	1	10	0.0125	0.2	0.0025
St. 2	10	0.250	10	0.250	1	10	0.025	0.2	0.0050
St. 3	25	0.250	10	0.625	1	10	0.063	0.2	0.012
St. 4	25	0.400	10	1.000	1	10	0.100	0.2	0.020
St. 5	200	0.075	10	1.500	1	10	0.150	0.2	0.030
St. 6	200	0.100	10	2.000	1	10	0.200	0.2	0.040
St. 7	200	0.125	10	2.500	1	10	0.250	0.2	0.050
St. 8	200	0.188	10	3.750	1	10	0.375	0.2	0.0750
St. 9	200	0.213	10	4.250	1	10	0.425	0.2	0.0850
LQC	200†	0.188	10	3.750	0.100	10	0.038	0.2	0.0075
MQC	200†	0.625	10	12.500	0.100	10	0.125	0.2	0.0250
HQC	200†	1.125	10	22.500	0.100	10	0.225	0.2	0.0450
Sample				0.5 g vegetation*		10		0.2	
IS‡				8.5	0.100	10	0.085	0.2	0.017

* Standards, quality controls, and matrix blanks were created using 0.5 g of untreated vegetation that was freeze-dried and homogenized with a ball-grinder for use in triclopyr LCMS/MS analysis. A 0.5 g vegetation sample of herbicide treated vegetation was also freeze-dried and homogenized with a ball-grinder for triclopyr LCMS/MS analysis.

† Prepare two separate 200 ug mL^{-1} stock solution for standards and quality controls.

‡Internal standard was added to all standards, quality controls, samples, and matrix blank before SPE and nitrogen evaporation.

Table B.5. Preparation of standards, quality controls, and samples for solid phase extraction for microcosm and avoidance sample triclopyr vegetation analysis.

St. and QC*	Stock 1 Conc. (ug/mL)	Stock 1 Vol. (mL)	Final Vol. (mL)	Stock 2 Conc. (ug/mL)	Stock 2 Vol. (mL)	Extr. Vol. (mL)	Extr. Conc. (ug/mL)	SPE Vol. (mL)	Final Conc. (ug/mL)
St. 1	10	0.125	10	0.125	0.5	5	0.013	0.2	0.0025
St. 2	10	0.250	10	0.250	0.5	5	0.025	0.2	0.005
St. 3	25	0.250	10	0.625	0.5	5	0.063	0.2	0.0125
St. 4	25	0.400	10	1.000	0.5	5	0.100	0.2	0.020
St. 5	200	0.075	10	1.500	0.5	5	0.150	0.2	0.030
St. 6	200	0.100	10	2.000	0.5	5	0.200	0.2	0.040
St. 7	200	0.125	10	2.500	0.5	5	0.250	0.2	0.050
St. 8	200	0.188	10	3.750	0.5	5	0.375	0.2	0.0750
St. 9	200	0.213	10	4.250	0.5	5	0.425	0.2	0.085
LQC	200†	0.188	10	3.750	0.05	5	0.038	0.2	0.0075
MQC	200†	0.625	10	12.500	0.05	5	0.125	0.2	0.025
HQC	200†	1.125	10	22.500	0.05	5	0.225	0.2	0.045
Sample				0.25 g vegetation*		5		0.2	
IS‡				8.5	0.05	5	0.085	0.2	0.017

* Standards, quality controls, and matrix blanks were created using 0.25 g of untreated vegetation that was freeze-dried and homogenized with a ball-grinder for use in triclopyr LCMS/MS analysis. A 0.25 g vegetation sample of herbicide treated vegetation was also freeze-dried and homogenized with a ball-grinder for triclopyr LCMS/MS analysis.

† Prepare two separate 200 ug mL^{-1} stock solution for standards and quality controls.

‡ Internal standard was added to all standards, quality controls, samples, and matrix blank before SPE and nitrogen evaporation.

B.5. Vegetation Sample Purification

Vegetation samples following treatment with Garlon XRT or Garlon RTU, standards, quality controls, and matrix blanks were purified via polymeric strong anion sorbent using separate Phenomenex Strata X (30 mg/1 mL) SPE cartridges by the following procedure (Table B.3):

a. Place separate Phenomenex Strata-X (30 mg/1 mL) SPE cartridges on vacuum manifold for each sample.

b. Condition SPE cartridge with 1.0 mL of acetonitrile followed by 1.0 mL of 1.0 N hydrochloric acid to equilibrate. Dry under full vacuumed between solvents.

c. Load sample solutions from Step B.4. – l. on to SPE cartridges. Draw samples through cartridges at a flow rate of approximately 0.5 mL min^{-1} and discard the eluate.

d. Rinse sample tube with 1.0 mL of 1N HCl and load onto the SPE cartridge. Discard the eluate.

e. Rinse SPE cartridge with 2 × 750 µL aliquots of acetonitrile/Milli-Q water/1N hydrochloric acid (30/69/1, v/v/v) solution at a flow rate of 1 mL min^{-1} and discard the eluate. Dry cartridge under full vacuum for 1 minute.

f. Elute triclopyr (and TCP) from SPE cartridges with 2 × 500 µL aliquots of acetonitrile/Milli-Q water/1 N hydrochloric acid (60/39/1, v/v/v) solution and collect eluate in 15 mL Falcon tubes. Dry SPE cartridges under full vacuum for 30 seconds after each aliquot.

g. Add 50 µL of 80:20 methanol:glycerol solution to each sample. Vortex for 30 seconds and centrifuge the samples for 5 minutes at 3500 rpm.

h. Evaporate the eluate to complete dryness using a TurboVap nitrogen evaporator set at 40 °C with nitrogen flow rate of approximately 15 psi.

i. Add 1.0 mL acetonitrile/Milli-Q water/1 N hydrochloric acid (60/39/1, v/v/v) solution to samples. Vortex to mix samples for 1 minute, followed by 10 minutes of sonication in warm water bath. Centrifuge the samples for 5 minutes at 3500 rpm. Note that samples below the lowest level of quantification were re-prepared and reconstituted in 0.5 mL acetonitrile/Milli-Q water/1 N hydrochloric acid (60/39/1, v/v/v) solution (instead of 1.0 mL).

j. Load half the purified samples into 2 mL glass autosampler vials and cap. Samples to be analyzed via LCMS/MS with the lowest level of quantification being 0.013 ug g $vegetation^{-1}$ (Equation B.2). The remaining sample half was stored in -20 °C. If samples were above standard concentration range, the following day stored samples were

diluting with a blank matrix and reanalyzed. Dilution integrity of 200-fold was assessed in method validation (Section B.7).

Equation B.2

$$\text{triclopyr in vegetation samples (ug g}^{-1}\text{)} = \text{extraction concentration} \times \frac{V1 \times Vf}{M \times V2}$$

where the extraction concentration is the concentration of standard 1 (0.0025 ug mL^{-1}), V1 is the initial extraction volume (5 mL), Vf is the final volume (0.5 mL), M is the sample weight (0.5 g vegetation), and V2 is the volume of aliquot (1.0 mL).

B.6. Mass Spectrometric Optimization of Triclopyr and Triclopyr [M+4]

High performance liquid chromatography coupled with tandem mass spectrometry (HPLC-MS/MS) analysis of triclopyr, stable isotope triclopyr [M+4] (internal standard), and TCP were conducted using an Agilent 1260 Infinity II HPLC system (Agilent Technologies, Santa Clara, CA, USA) equipped with AB Sciex 4000 hybrid triple quadrupole linear ion trap mass spectrometry (AB Sciex, Concord, ON, Canada) and a Turbo V™ Ion Spray electrospray ionization (ESI) source using nitrogen as the collision gas. The HPLC contains a binary pump with an autosampler and temperature controlled at 4 °C. The optimization of triclopyr and triclopyr [M+4] were conducted in negative ion mode, where the collisional energy varied between -22.0 V and -10.0 V, the declustering potential was between -60.0 V and -80.0 V, and dwell time for all transitions was 100 ms at unit resolution. The theoretical ion mass-to-charge (m/z, Da) peak chromatograph of triclopyr in the negative ion mode is displayed in Figure B.1. The concentration of triclopyr was determined by reporting the chromatographic peak areas of the samples (peak area ratio of the analyte to internal standard) by the linear regression of the standard solution concentrations using AB Sciex Analyst® Software version 1.6.2 (SCIEX. 2013. Analyst 1.6.2 Software Installation Guide. Framingham, MA, USA).

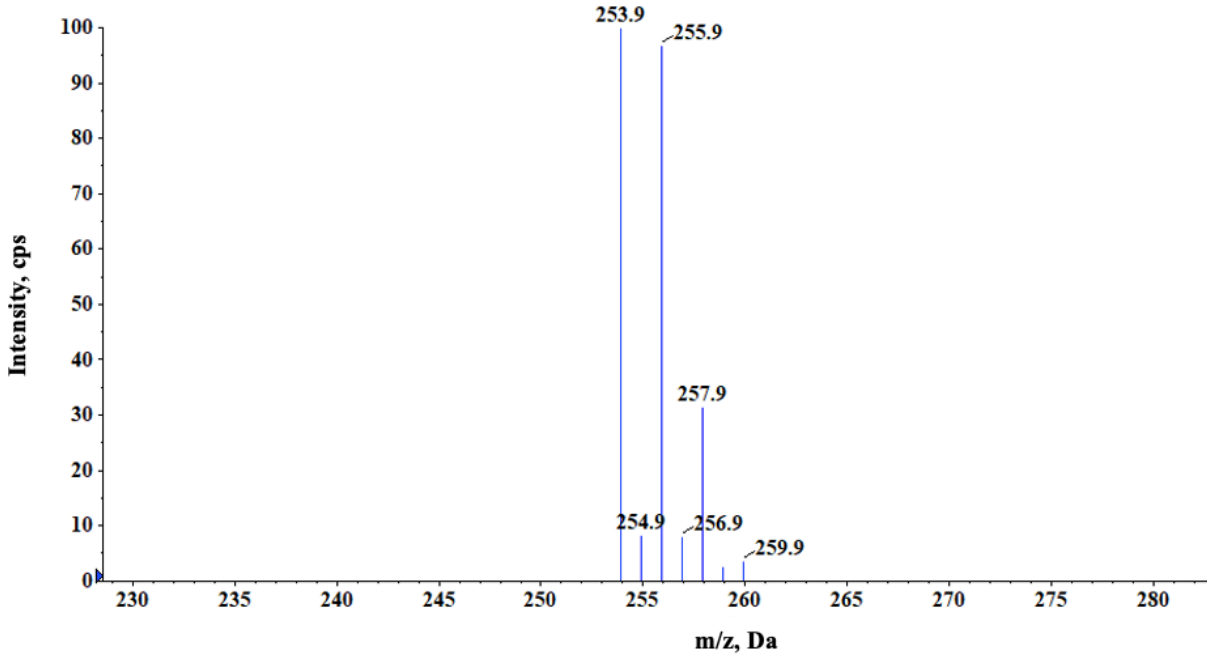

Figure B.1. Theoretical ion mass-to-charge (*m/z*, Da) chromatogram of triclopyr in negative ion mode.

The fractionation pattern, product ions, and MS conditions for triclopyr and triclopyr [M+4] were identified through triclopyr injection into the MS (Table B.6). The transitions monitored and used for the quantification for triclopyr were m/z 253.8 (precursor ion) → m/z 195.7 (product ion) and m/z 261.8 → m/z 203.8 for triclopyr [M+4] stable isotope used for the internal standard (Figure B.1 and B.2). The other transitions monitored for triclopyr (m/z 253.8 → m/z 217.7 and m/z 255.7 → m/z 197.8 and m/z 219.8) and the internal standard m/z 261.8 → m/z 179.8, m/z 223.7, and m/z 225.6) were used for analyte qualification (Figures B.1, B.2, and B.3). The transitions were determined by injecting an infused sample into the mass spectrometer with an isocratic mobile phase of methanol/acetonitrile (50:50, v/v) with 0.1% acetic acid for a 7-minute run time. Multiple reaction monitoring (MRM) was accomplished through electrospray ionization (ESI) source with nitrogen as the nebulizing gas in the negative ion mode. The optimized chromatographic and instrumental parameters for triclopyr and triclopyr [M+4] quantification on the HPLC-MS/MS are in Table B.7.

Table B.6. HPLC-MS/MS quantifying parameter for triclopyr and triclopyr [M+4] stable isotope: where Q1 is the quantifier and qualifier precursor ion, Q3 is the quantifier and qualifier product ions, DP is the declustering potential, EP is the entrance potential, CE is the collision energy, CXP is the collision cell exit potential, and amu represents the atomic mass unit (Da). Bolded Q1 and Q3 transitions indicate use for quantification.

ID	Retention Time	Q1	Q3	DP	EP	CE	CXP
	min	amu		volts			
Ticlopyr	3.20 (Drift XRT)	255.7	197.8	-60	-10	-16	-5
			219.8	-60	-10	-10	-5
		253.8	**195.7**	-60	-10	-18	-3
	3.33 (Drift RTU)		217.7	-60	-10	-10	-5
Triclopyr [M+4]	3.30 (Vegetation)	**261.8**	179.8	-80	-10	-22	-13
			203.8	-80	-10	-18	-5
			223.7	-80	-10	-10	-5
			225.6	-80	-10	-10	-5

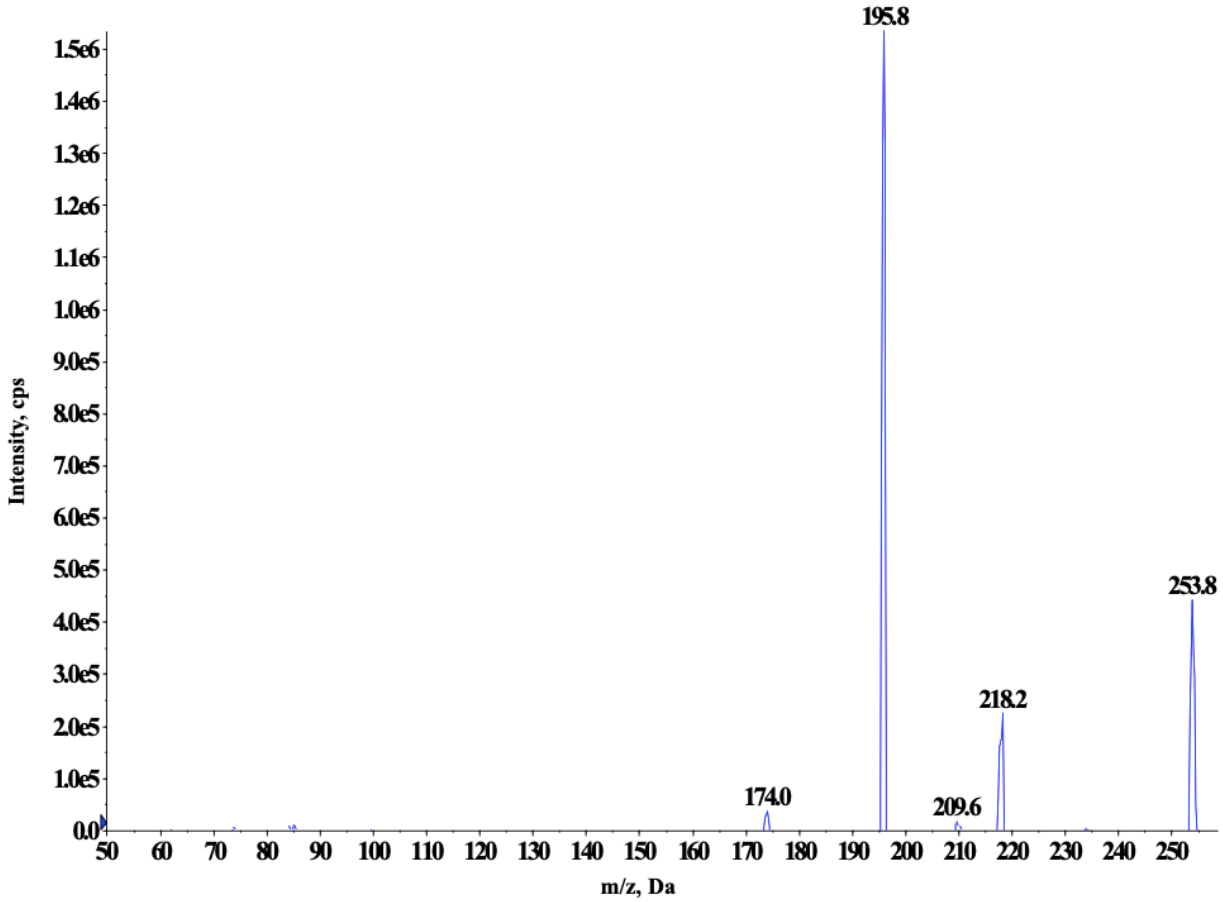

Figure B.2. Transitions chromatogram of triclopyr precursor ion, *m/z* 253.8, and its fractionation product ions: *m/z* 253.8 (quantification) → *m/z* 195.7 (quantification), *m/z* 217.7 (qualification).

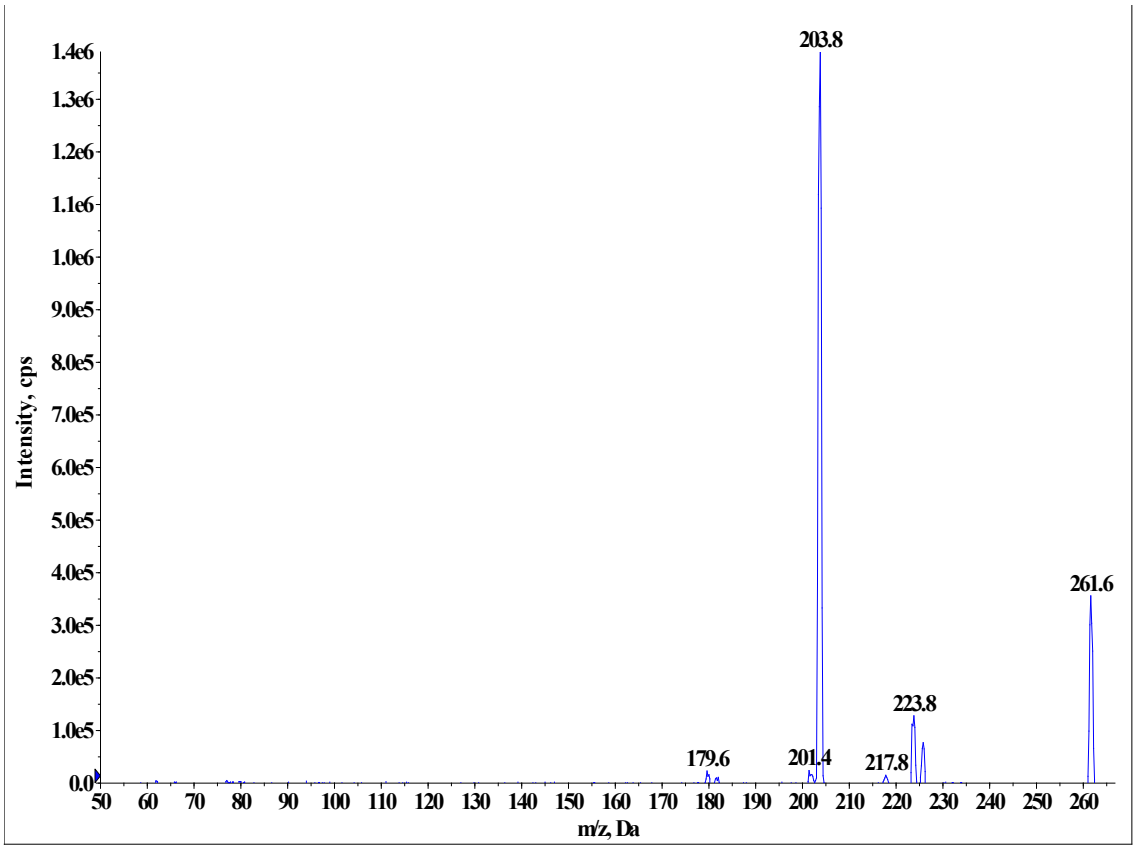

Figure B.3. Transitions chromatogram of triclopyr [M+4] stable isotope used for the internal standard precursor ion, *m/z* 261.6, and its fractionation product ions: *m/z* 261.8 (quantification) → *m/z* 203.8 (quantification), *m/z* 179.8 (qualification), *m/z* 223.7 (qualification), and *m/z* 225.6 (qualification).

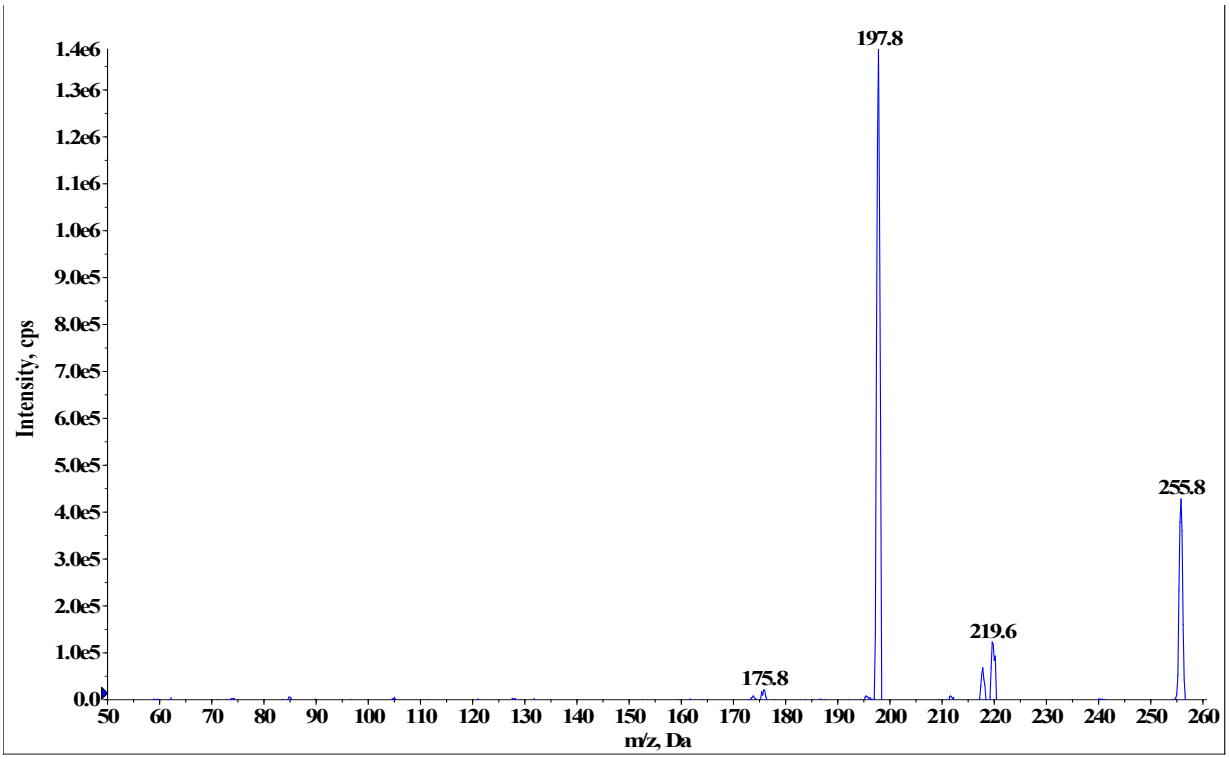

Figure B.4. Transitions chromatogram of triclopyr precursor ion, *m/z* 255.7, and its fractionation product ions: *m/z* 255.7 (qualification) → *m/z* 197.8 (qualification), *m/z* 219.8 (qualification).

Table B.7. LCMS/MS Instrument conditions for the quantification of triclopyr and triclopyr [M+4] stable isotope uses as the internal standard.

Mass Spectrometer	AB SCIEX 4000 QTRAP HPLC-MS/MS System		
HPCL Instrument	Agilent 1260 Infinity II HPCL System		
Analytical Column	Agilent Poroshell 120 EC-C18, 4.6 x 50 mm, 2.7 um, 120 A		
Column Temperature (°C)	40 ± 0.05		
Injection Volume (uL)	10		
Run Time (min)	7.0		
Flow Rate (mL min^{-1})	0.5		
Mobile Phase (A)	0.1% Acetic Acid in Water		
Mobile Phase (B)	0.1% Acetic Acid in Acetonitrile		
Mobile Phase Gradient	Time (min)	Solvent A (%)	Solvent B (%)
	0	60	40
	4	10	90
	4.5	50	50
Needle Rinsing Solvent	Acetonitrile:Water (70:30, v/v)		
Interface	Electrospray Ionization (ESI)		
Polarity	Negative		
Scan Type	Multiple Reaction Monitoring		
Curtain Gas (psi)	30		
Collision Gas (psi)	10		
Ion Spray Voltage (V)	-4500		
Interface Temperature (°C)	500		
ESI Nebulizing Gas	Nitrogen		
Ion Source Gas 1 (psi)	50		
Ion Source Gas 2 (psi)	50		

Analyzing the metabolite of triclopyr, TCP, was optimized on the HPLC-MS/MS using the equipment and procedure described above. The optimization of TCP was conducted in the positive ion mode, where the collisional energy varied between 30.0 V and 42.0 V, the declustering potential was 38.0 V, and dwell time for all transitions was 50 ms at unit resolution. The fractionation pattern, product ions and MS conditions for TCP were identified through TCP injection into the MS (Table B.8). The transitions monitored for TCP were m/z 198.0 (quantifier) → m/z 107.0 (quantitative) and m/z 134.0 (qualitative). The transitions for TCP were determined

by injecting an infused sample into the mass spectrometer with an isocratic mobile phase of acetonitrile with 0.1% formic acid for a 7-minute run time. Multiple reaction monitoring (MRM) was achieved through electrospray ionization (ESI) source with nitrogen as the nebulizing gas in the positive ion mode. The optimized chromatographic and instrumental parameters for TCP quantification on the HPLC-MS/MS are in Table B.9. No TCP was detected in the leaves of *Populus tremuloides* or *Salix bebbiana*, so further method development ceased.

Table B.8. HPLC-MS/MS quantifying parameter for TCP: where Q1 is the quantifier precursor ion, Q3 is the quantifier product ions, DP is the declustering potential, EP is the entrance potential, CE is the collision energy, CXP is the collision cell exit potential, and amu represents the atomic mass unit (Da).

ID	Retention Time	Q1	Q3	DP	EP	CE	CXP
	min	———amu———		———————volts———————			
			107.0	38	10	42.0	18.7
TCP	2.97	198.0	134.0	38	10	32.5	24.9
			134.0	38	10	30.0	6.5

Table B.9. HPLC-MS/MS Instrument conditions for the quantification of TCP.

Mass Spectrometer	AB SCIEX 4000 QTRAP HPLC-MS/MS System		
HPCL Instrument	Agilent 1260 Infinity II HPCL System		
Analytical Column	Agilent Poroshell 120 EC-C18, 4.6 x 50 mm, 2.7 um, 120 A		
Column Temperature (°C)	40 ± 0.05		
Injection Volume (uL)	10		
Run Time (min)	7.0		
Flow Rate (mL min^{-1})	0.5		
Mobile Phase (A)	0.1% Formic Acid in Water		
Mobile Phase (B)	0.1% Formic Acid in Methanol:Acetonitrile (50:50, v/v)		
Mobile Phase Gradient	Time (min)	Solvent A (%)	Solvent B (%)
	0	60	40
	4	10	90
	4.5	50	50
Needle Rinsing Solvent	Acetonitrile:Water (70:30, v/v)		
Interface	Electrospray Ionization (ESI)		
Polarity	Positive		
Scan Type	Multiple Reaction Monitoring		
Curtain Gas (psi)	30		
Collision Gas (psi)	10		
Ion Spray Voltage (V)	5500		
Interface Temperature (°C)	500		
ESI Nebulizing Gas	Nitrogen		
Ion Source Gas 1 (psi)	50		
Ion Source Gas 2 (psi)	50		

B.7. METHOD VALIDATION

The method validation for the detection of triclopyr in both Garlon XRT and Garlon RTU drift and vegetation samples followed the FDA Guidelines for Bioanalytical Method Validation (USFDA 2018) as described by Michel et al. (2015), including selectivity, linearity, precision, accuracy, reproducibility, recovery (vegetation samples), and matrix effects (vegetation samples). The concentration of triclopyr was determined by reporting the chromatographic peak areas (peak area ratio of the analyte to internal standard) of the samples versus standard solution concentrations. The concentrations used for the standard solutions were fit for purpose. A linear regression analysis weighing the standard curve was not performed for drift samples; however, a

weighing of the standard curve with 1/x was conducted for the analysis of triclopyr concentration in vegetation. Standard curve passed validation if the calibration curve correlation coefficient was $r \geq 0.98$ and at least six concentrations were within 15% of nominal values. The lowest detectable concentration for triclopyr was considered environmentally irrelevant to determine on the HPLC-MS/MS, as the signal-to-noise ratio of the lower limit of quantification (LLOQ) was above 30, and often magnitudes above. The accuracy and precision of LLOQ was accepted if within 20% of the nominal value and the coefficient of variation (CV) for intra and inter-day samples were within 10%.

The accuracy and precision of the standard curves were assessed through replicate sets of quality control (QC) samples spiked independently of calibration standards. The LLOQ, low quality control (LQC), medium quality control (MQC), and high quality control (HQC) were prepared in replicates and quantified on the HPLC-MS/MS with standard solution concentrations every day for three days, with the first day of HPLC-MS/MS validation analysis reanalyzed on the second day to ensure stability. Accuracy and precision of QCs were accepted if the concentrations of at least five replicates within an analysis were 15% of the nominal value and the coefficient of variation (CV) for intra and inter-day samples were within 10%. Validation parameters for Garlon XRT drift samples are displayed in Table B.10 (intra-day) and Table B.11 (inter-day). Drift samples from Garlon RTU appeared to quantify on the HPLC-MS/MS similar to drift samples from Garlon XRT and therefore were only validated over one day, having met all validation parameters described above (Table B.12). Validation parameters for triclopyr concentration in vegetation are displayed in Table B.13 (intra-day) and Table B.14 (inter-day). Matrix effects were also evaluated in method validation for triclopyr in vegetation by spiking the matrix with the same concentration before and after extraction (via solid phase extraction) and spiking solvent with the same concentration (without matrix or extraction) in replicates of six. All replicates were within 15% of nominal concentrations and had %CVs below 5%. Dilution integrity was also assessed for vegetation samples by spiking a matrix blank with a known concentration, then diluting the samples 200-fold following extraction with a blank matrix. All six replicates were within 15% of the nominal concentration and had %CVs below 5%.

Table B.10. Intra-day accuracy and precision for validation of Garlon XRT drift.

Quality Control	Replicates	Analysis Day (#)	Observed Concentration (mean ± SD; ng mL^{-1})	Precision (%CV)	Accuracy (%)
LLOQ (6 ng mL^{-1})	6	1	5.744 ± 0.251	4.4	100.7
	6	2	6.173 ± 0.186	3.0	103.0
	6	3	5.412 ± 0.220	4.1	90.2
LQC (18 ng mL^{-1})	5	1	18.379 ± 0.237	1.3	102.1
	6	2	19.241 ± 0.265	1.4	106.9
	6	3	17.053 ± 0.795	4.7	94.7
MQC (36 ng mL^{-1})	6	1	35.878 ± 0.454	1.3	99.7
	6	2	37.087 ± 0.882	2.4	103.0
	6	3	37.350 ± 1.143	3.1	103.8
HQC (51 ng mL^{-1})	6	1	49.454 ± 2.009	4.1	97.0
	5	2	51.496 ± 0.443	0.9	101.0
	6	3	53.512 ± 0.846	1.6	104.9

Table B.11. Inter-day accuracy and precision for validation of Garlon XRT drift.

Quality Control	Concentration (ng mL^{-1})	Replicates	Observed Concentration (mean ± SD; ng mL^{-1})	Precision (%CV)	Accuracy (%)
LLOQ	6	17	0.578 ± 0.400	6.9	96.4
LQC	18	17	18.218 ± 1.072	5.9	101.9
MQC	36	18	37.100 ± 1.177	3.2	103.1
HQC	51	17	51.488 ± 2.143	4.2	101.0

Table B.12. Intra-day accuracy and precision for validation of Garlon RTU drift.

Quality Control	Concentration (ng mL^{-1})	Replicates	Observed Concentration (mean ± SD; ng mL^{-1})	Precision (%CV)	Accuracy (%)
LLOQ	6	5	6.566 ± 0.699	10.6	106.1
LQC	18	5	16.540 ± 0.850	5.1	91.8
MQC	36	6	32.433 ± 0.677	2.1	90.1
HQC	51	5	44.640 ± 0.902	2.0	87.5

Table B.13. Intra-day accuracy and precision for validation of triclopyr vegetation concentration.

Quality Control	Reps.	Analysis Day (#)	Observed Concentration (mean ± SD; ng mL^{-1})	Precision (%CV)	Accuracy (%)
LLOQ (2.5 ng mL^{-1})	5	1	2.350 ± 0.146	2.8	94.2
	5	2	2.504 ± 0.183	7.3	100.1
	5	3	2.388 ± 0.122	5.1	95.4
LQC (7.5 ng mL^{-1})	5	1	7.310 ± 0.318	4.4	97.4
	6	3	7.291 ± 0.231	3.2	97.1
	5	4	7.100 ± 0.196	2.8	94.6
MQC (25.0 ng mL^{-1})	6	1	24.383 ± 0.549	2.3	97.2
	6	2	24.133 ± 0.498	2.0	96.5
	6	3	23.850 ± 0.404	1.7	95.4
HQC (45.0 ng mL^{-1})	6	1	41.467 ± 1.413	3.4	92.2
	6	2	41.507 ± 0.897	2.2	92.3
	6	3	41.983 ± 0.867	2.1	93.3
Post-Extraction Spike (25.0 ng mL^{-1})	6				
Pre-Extraction Spike (25.0 ng mL^{-1})	6	3	22.86 ± 0.990	4.3	94.4
No Matrix Spike (25.0 ng mL^{-1})	6				
Dilution Integrity (200x) (40.0 ng mL^{-1})	6	4	44.484 ± 1.825	4.1	111.2

Table B.14. Inter-day accuracy and precision for validation of triclopyr vegetation concentration.

Quality Control	Concentration (ng mL^{-1})	Replicates	Observed Concentration (mean ± SD; ng mL^{-1})	Precision (%CV)	Accuracy (%)
LLOQ	2.5	17	2.401 ± 0.146	6.1	96.0
LQC	7.5	17	7.294 ± 0.339	4.6	97.2
MQC	25.0	18	24.122 ± 0.507	2.1	96.5
HQC	45.0	18	41.650 ± 1.051	2.5	92.6

Sensitivity, carry-over, extraction recovery, and the matrix factor were assessed for the validation of methods to determine triclopyr when applicable. The sensitivity of the methods was evaluated by comparing the area of the analyte or internal standard (IS) of a matrix blank to that of the LLOQ (Equations B.3 and B.4) and confirmed to be less than 20% (analyte) and 5% (IS). Both methods to evaluate triclopyr from Garlon XRT or Garlon RTU drift were sensitive. The matrix blank peak chromatogram for the Garlon XRT drift method is displayed in Figure B.5 and the peak chromatogram of standard six (of eight) is shown in Figure B.6. The matrix blank peak chromatogram for the Garlon RTU drift method is displayed in Figure B.7 and the peak chromatogram of standard six (of eight) is shown in Figure B.8. Contamination was found to be in the matrix blank for the method to determine triclopyr in vegetation (willow), but concentrations were below the lowest level of quantification and satisfied standards for sensitivity. The matrix blank peak chromatogram for the method of determining triclopyr in vegetation is displayed in Figure B.9 and the peak chromatogram of standard six (of nine) is shown in Figure B.10. Minimal triclopyr carry-over was observed between samples of high (ie. upper limit of quantification (ULOQ)) to low concentration (ie. LLOQ) and was verified using Equations B.3 and B.4. Carry-over results for all validated methods were less than 20% (analyte) and 5% (IS). Extraction recovery and the matrix factor were determined for the method of validating of triclopyr in vegetation by Equations B.7 and B.8, respectively. The extraction recovery was 87% and the matrix factor was 72%. Method validation results for sensitivity, carry-over, extraction recovery, and the matrix factor are summarized in Table B.15.

Equation B.3

$$\text{sensitivity (\%)} = \frac{\text{analyte peak area in matrix blank}}{\text{analyte peak area in LLOQ}} \times 100 \leq 20\%$$

Equation B.4

$$\text{sensitivity (\%)} = \frac{\text{internal standard peak area in matrix blank}}{\text{internal standard peak area in LLOQ}} \times 100 \leq 5\%$$

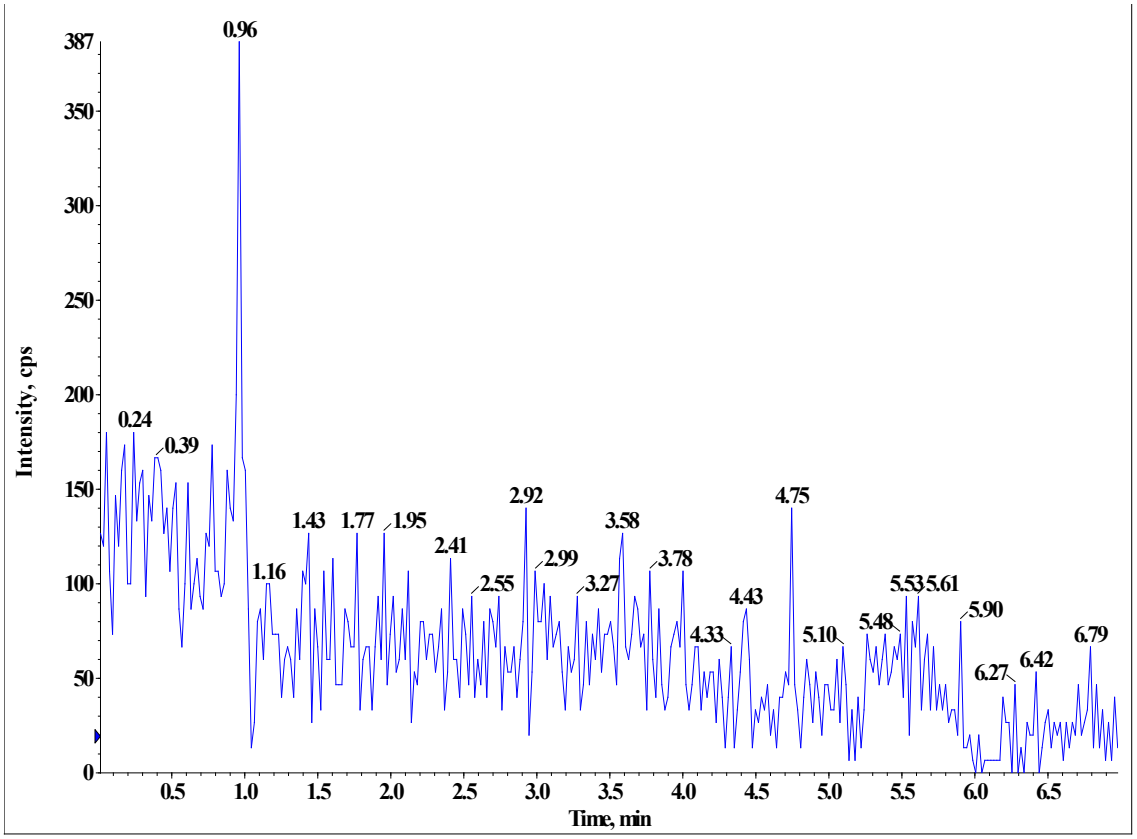

Figure B.5. Peak chromatogram for matrix blank from Garlon XRT drift validation.

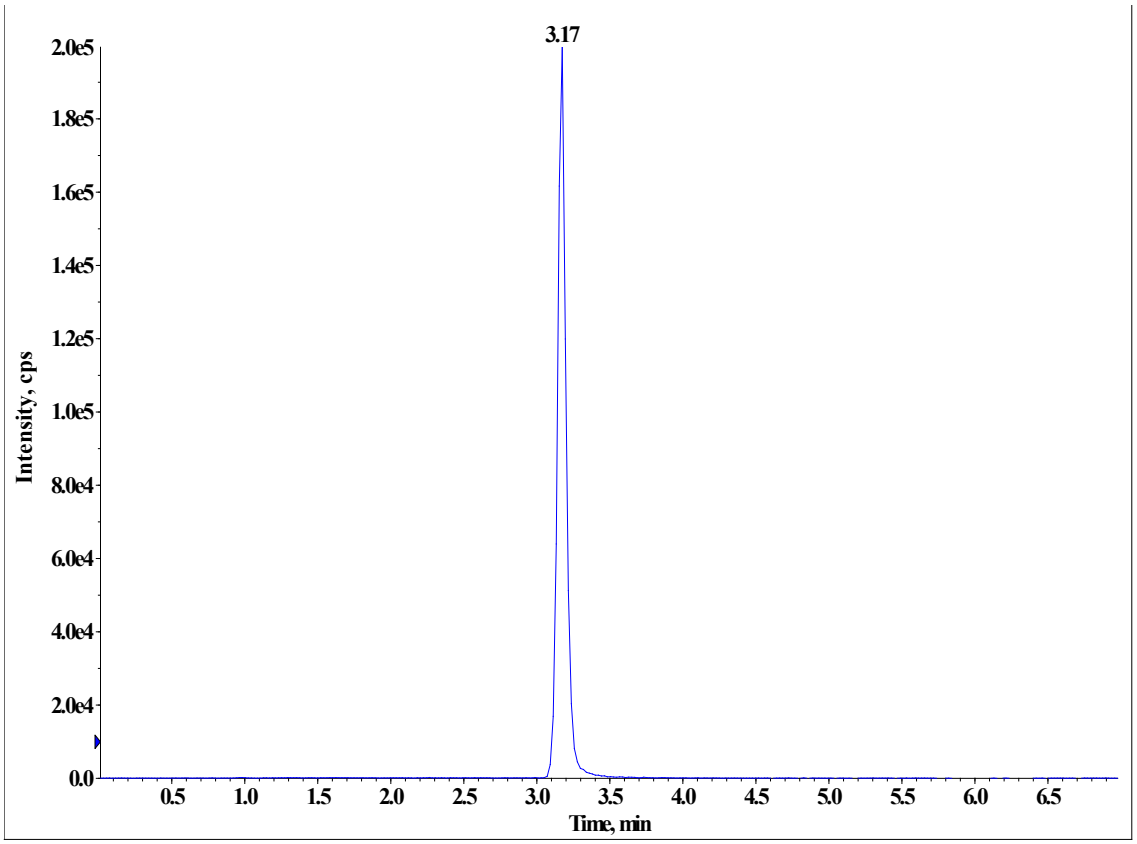

Figure B.6. Peak chromatogram for standard six from Garlon XRT drift validation.

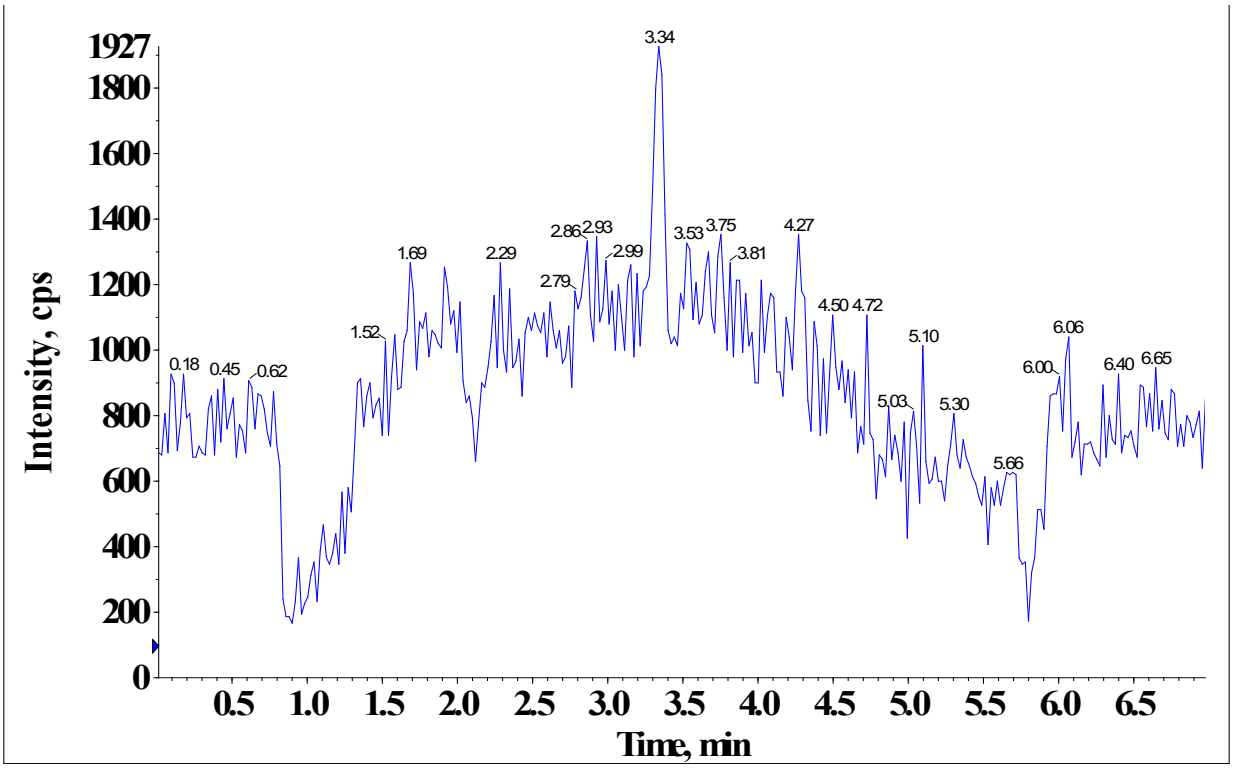

Figure B.7. Peak chromatogram for matrix blank from Garlon RTU drift validation.

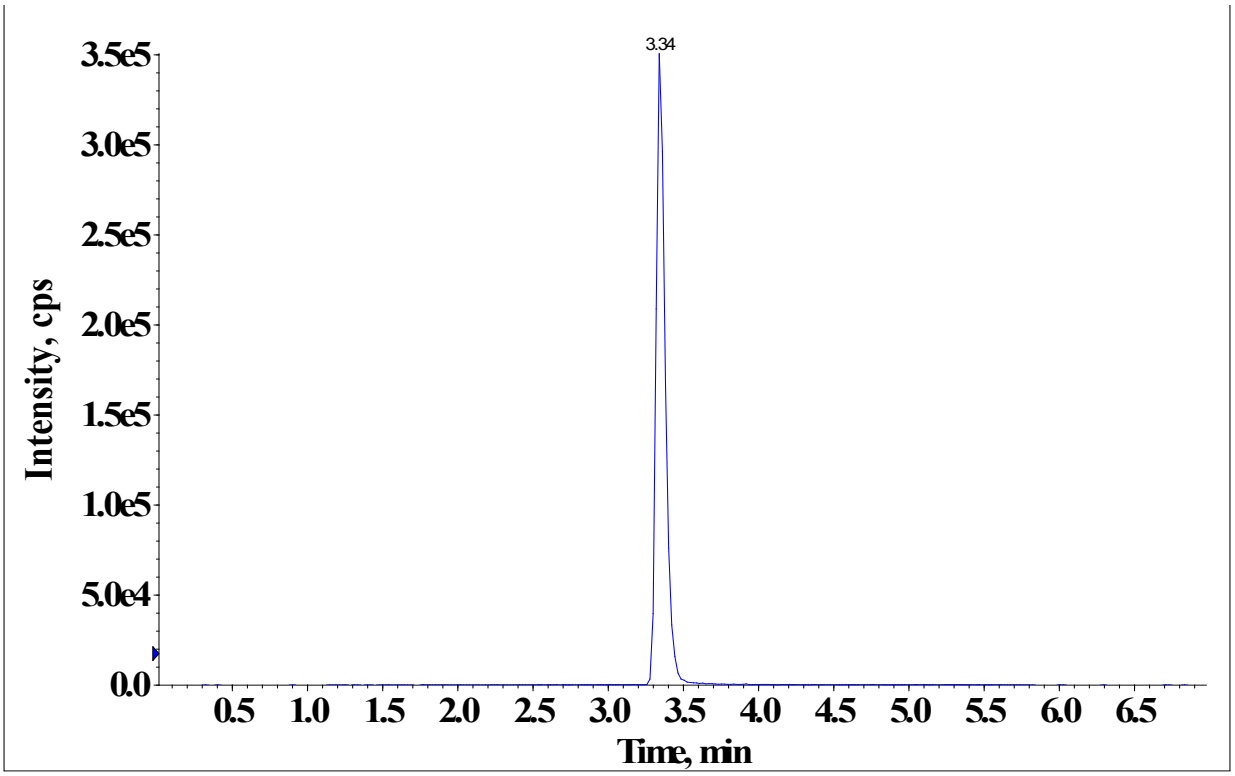

Figure B.8. Peak chromatogram for standard six from Garlon RTU drift validation.

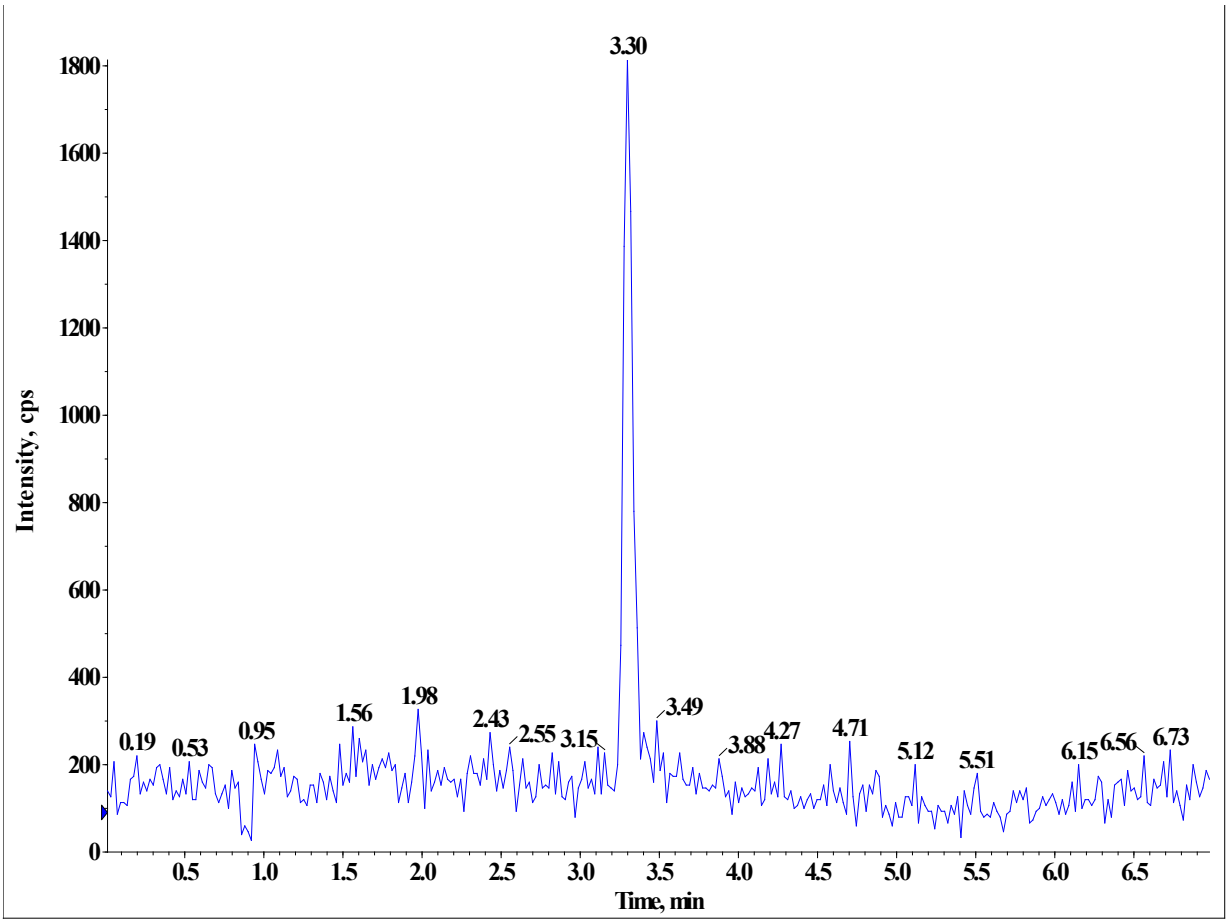

Figure B.9. Peak chromatogram for matrix blank from validation for triclopyr in vegetation.

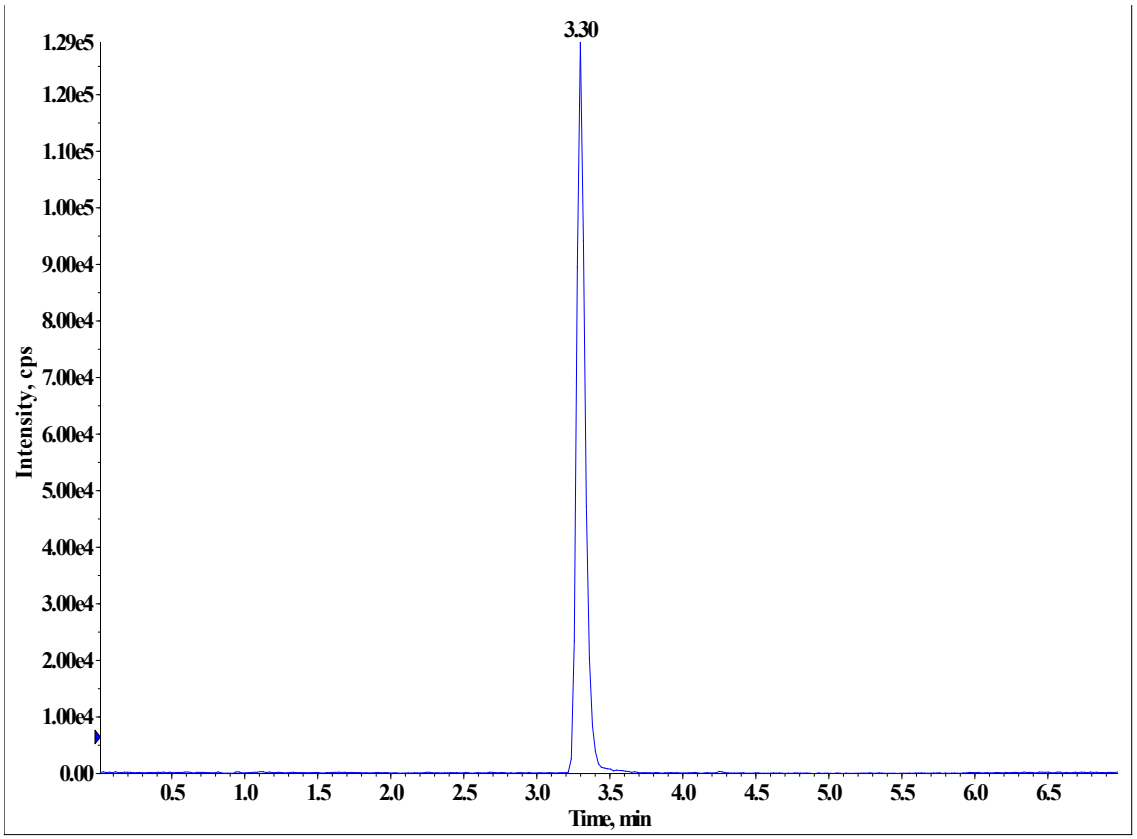

Figure B.10. Peak chromatogram for standard six from validation for triclopyr in vegetation.

Equation B.5

$$\text{carry-over (\%)} = \frac{\text{analyte peak area in blank after ULOQ}}{\text{analyte peak area in LLOQ}} \times 100 \leq 20\%$$

Equation B.6

$$\text{carry-over (\%)} = \frac{\text{internal standard peak area in blank after ULOQ}}{\text{internal standard peak area in LLOQ}} \times 100 \leq 5\%$$

Equation B.7

$$\text{extraction recovery (\%)} = \frac{\text{analyte peak area of pre SPE spike}}{\text{analyte peak area of post SPE spike}} \times 100$$

Equation B.8

$$\text{matrix factor (\%)} = \frac{\text{analyte peak area of post SPE spike}}{\text{analyte peak area of pure solvent spike}} \times 100$$

Table B.15. Method validation results for sensitivity, carry-over, extraction recovery, and the matrix factor.

Method	Sensitivity (%)		Carry-over (%)		Extraction Recovery (%)	Matrix Factor (%)
	Analyte ≤ 20%	IS ≤ 5%	Analyte ≤ 20%	IS ≤ 5%		
Drift XRT	0.32 ± 0.11	0.47 ± 0.15	0.78 ± 0.18	0.33 ± 0.04	N/A	N/A
Drift RTU	0.27	0.10	0.38	0.05	N/A	N/A
Vegetation	14.98 ± 4.33	0.23 ± 0.03	1.89 ± 0.88	0.60 ± 0.07	87	72

Every sample quantification HPLC-MS/MS analysis contained at least six calibration points detected within 15% of nominal concentration, except for the LLOQ, which was within 20% as per guidelines. At least 67% of QCs (and 50% of QC at each concentration) were within 15% of the nominal value. The calibration curve correlation coefficient was $r \geq 0.098$. Each HPLC-MS/MS analysis contained a pure solvent solution, matrix blank, matrix blank with internal standard, calibration standards (at least six), and QCs (making up at least 5% of the total unknown samples).

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APPENDIX C: TRICLOPYR DISSIPATION IN VEGETATION MODELLED WITH WINTER BREAK

LIST OF FIGURES

C.1. STATISTICAL ANALYSIS

Triclopyr residues in willow (*Salix bebbiana*) leaves from a foliar treatment of Garlon XRT were evaluated at six time points until winter (90 DAT) (n = 5 per time point), as well as at four time points after winter (280 DAT) until a year after treatment (n = 5 per time point). Herbicide concentrations were natural log transformed. Models were best explained by a first order kinetic linear regression. The models were examined to confirm assumptions of homoscedasticity of model variance (Non-Constant Variance Test, $p>0.05$) and normality of residuals (Shapiro Wilk Normality Test, $p>0.05$). Statistical analyses were completed and plotted using the R software (version 3.6.1) (R Core Team 2019). Dissipation of triclopyr residues was determined for both the time it took residues to dissipate to 50% of the initial concentration 50% (DT_{50}) in willow leaves using the data modelled from 0 to 90 DAT and the time it took residues to dissipate to 10% of the initial concentration (DT_{90}) using the data modelled from 280 to 365 DAT. The DT_{50} and DT_{90} values were determined using the following first order kinetic equation:

Equation C.9

$$C_t = -k\mathrm{t} + C_0$$

where C_t is the herbicide concentration in leaves at time t, k is the dissipation rate constant of the kinetics model, and C_0 is the herbicide concentration at time zero.

Note that triclopyr residues in leaves from a basal bark treatment of Garlon RTU in aspen (*Populus tremuloides*) saplings were evaluated at eight time points between two days when maximum concentrations were reached (estimate) and winter (91 DAT) (n = 5 per time point, with the exception of 59 DAT where n = 4). There were only two time points following the winter break, so dissipation was not modelled for this time period. In addition to poorly explaining the variably in the data ($r^2 = 0.105$), the model did not meet assumptions of homoscedasticity of model variance (Non-Constant Variance Test, $p>0.05$) or normality of residuals (Shapiro Wilk Normality Test, $p>0.05$).

C.2. RESULTS

Dissipation in leaves of *S. bebbiana* shrubs treated with Garlon XRT (triclopyr) via low-volume foliar application followed first order dissipation kinetics. The first model describes the data from time zero to the last sampling point before winter, 90 DAT (Figure C.1). The degradation constant ($k = -0.023$, $p = \leq 0.05$) obtained from the linear regression equation was used to determine the DT_{50} as per the Equation C.1, which was resolved to be 29.9 DAT (Figure C.1). The second model describes the data from the first sample point after winter (280 DAT) until a year after treatment (Figure C.2). The degradation constants ($k = -0.1682$, $p = \leq 0.05$) obtained from the linear regression equation was used to determine the DT_{90} as per the Equation C.1, which was resolved to be 191.8 DAT (Figure C.2). These models were a poor fit to the observed data. The data was best explained modelled from 0 to 365 DAT by a hockey stick linear regression consisting of two sequential first order kinetic models with the breaking point at 16.1 DAT ($r^2 = 0.818$) (Section 4.3.2, Figure 4.9).

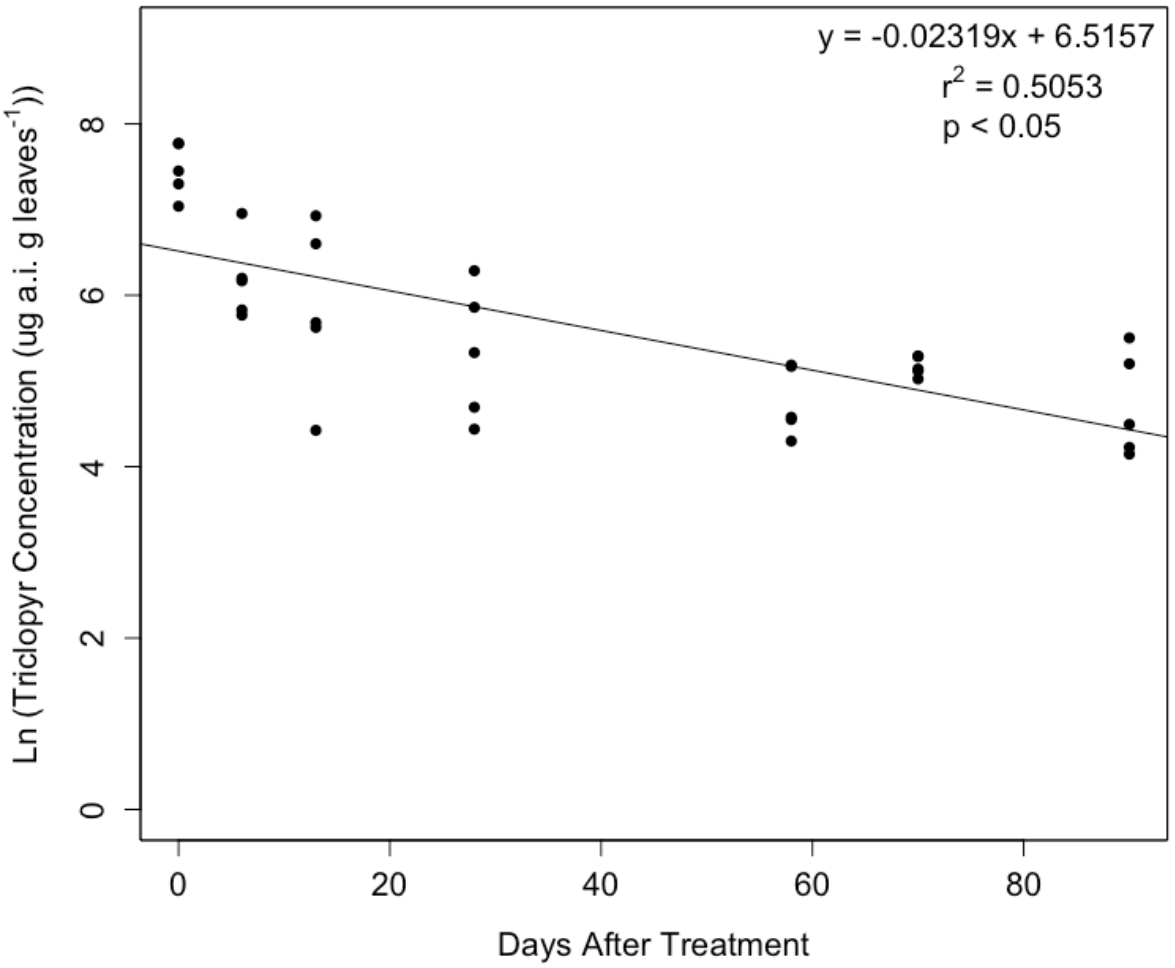


Figure C.1. First order dissipation model for triclopyr dissipation in the leaves of *Salix bebbiana* shrubs following a low-volume foliar application of Garlon XRT from 0 to 90 DAT, the last sampling point before winter (r^2 = 0.5053). The DT_{50} and was calculated as 29.9 DAT.

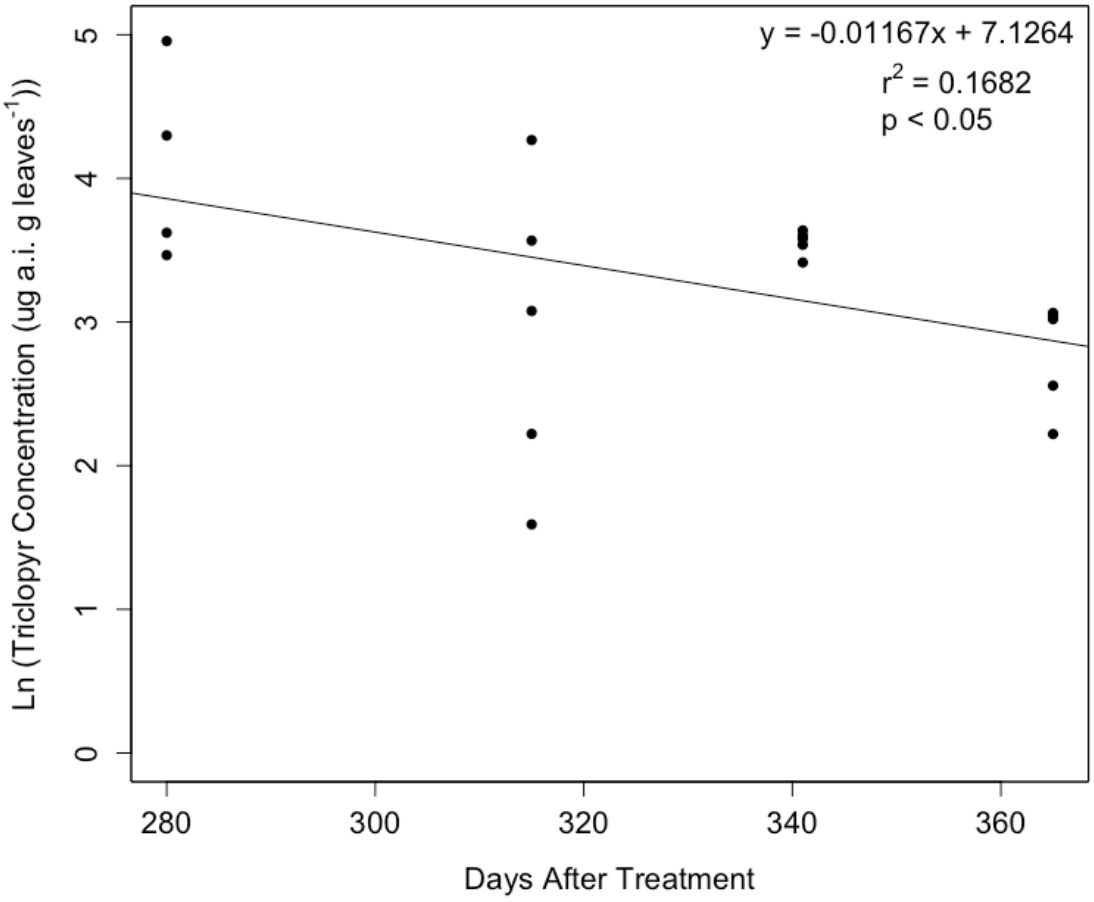


Figure C.2. First order dissipation model for triclopyr dissipation in the leaves of *Salix bebbiana* shrubs following a low-volume foliar application of Garlon XRT from after winter (280 DAT) until a year following application ($r^2 = 0.1682$). The DT_{90} and was calculated as 191.8 DAT.

Dissipation in leaves of *P. tremuloides* saplings treated with Garlon RTU (triclopyr) via basal bark application were modelled between two days when maximum concentrations were reached (estimate) and winter (91 DAT) using first order dissipation kinetics. This model was a poor fit for the observed dissipation data ($r^2 = 0.105$) and did not meet assumptions of homoscedasticity of model variance or normality of residuals (Figure C.3). The data was better described by a first order dissipation kinetics model ($r^2 = 0.26$) (Section 4.3.2, Figure 4.8), although dissipation times were not estimated due to low explanatory power of the linear model.

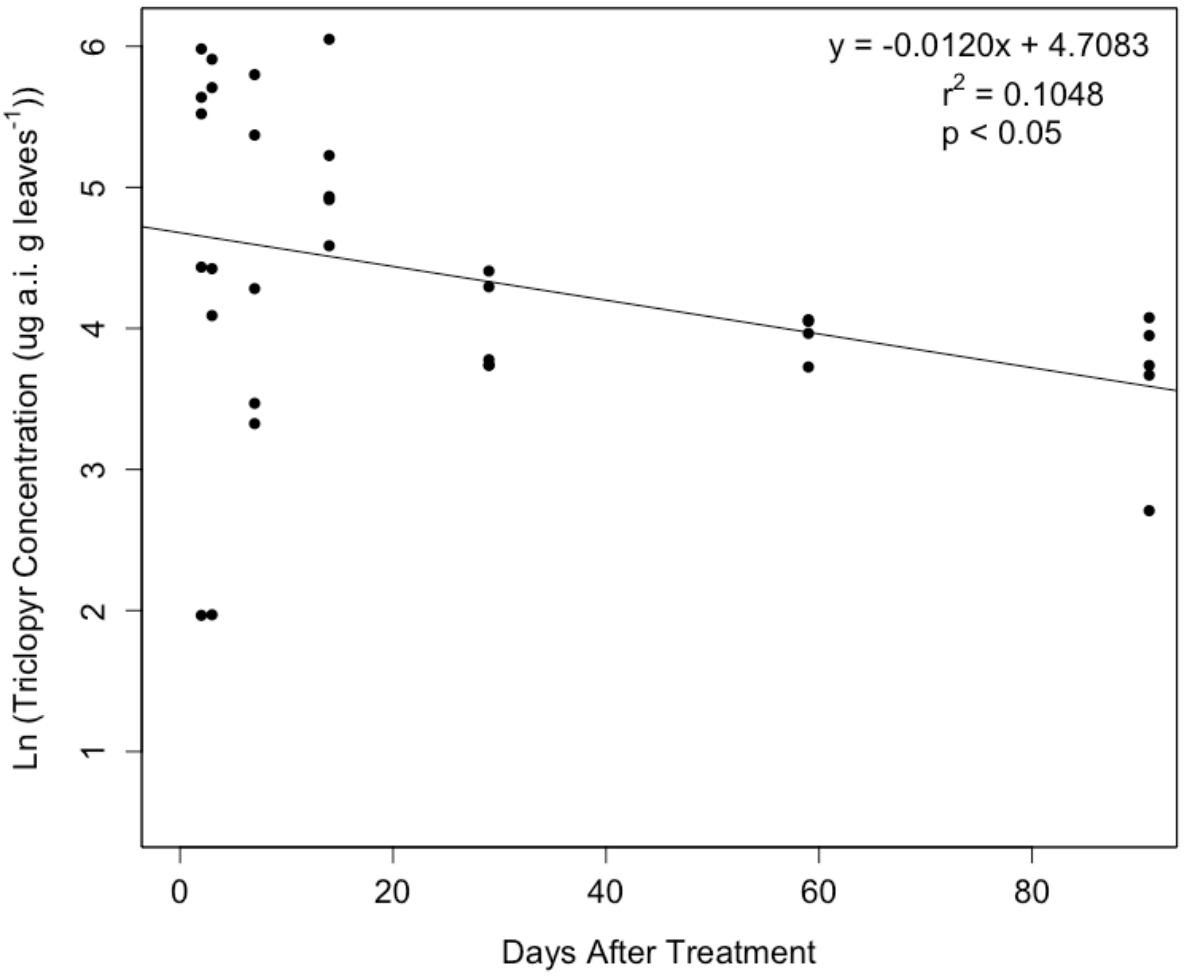


Figure C.3. First order dissipation model for triclopyr dissipation in the leaves of *Populus tremuloides* saplings following a basal bark treatment of Garlon RTU from two days when concentrations were highest (estimate) to 91 DAT, the last sampling point before winter (r^2 = 0.105). Model did not meet assumptions of homoscedasticity of model variance (Non-Constant Variance Test, p>0.05) or normality of residuals (Shapiro Wilk Normality Test, p>0.05).

References:

R Core Team. 2019. R: A Language and Environment for Statistical Computing. R version 3.6.1 (Action of the Toes). R Foundation for Statistical Computing, Vienna, Austria.

APPENDIX D: LITTERBAG 28 DAT

LIST OF FIGURES

Figure D.1. Litter breakdown of willow leaves (*S. bebbiana*) buried in the field litterbags at 28 days after a low-volume foliar application of Garlon XRT (triclopyr). Mass loss expressed as a percentage (initial dry wt-final dry wt)/initial dry weight) and litter bags removed at four time points over a year. Different letters indicate a significant difference in mass loss over time (ANOVA, TukeyHSD<0.05).

D.1. STUDY DESIGN

Leaf litter breakdown was examined at the Willow site employing the pervious study design described in Section 4.2.1, 4.2.2. (4.2.2.3) and 4.2.3 (Figure 4.4). Leaves from 20 treated and 24 untreated *Salix bebbiana* shrubs were collected from the Willow site and compiled into two respective homogenous composites 28 days following herbicide application. Four grams ± 0.01 of desiccated leaves from the treated leaf composite and 7 ± 0.01 g of fresh leaves from the untreated leaf composite were placed in a 1.3 mm^2 nylon mesh litterbags (15 x 15 x 1 cm) (Chomel et al. 2016). Since herbicide residue in the leaves may be effected by drying, litter was not dried prior to placement in the litterbags and the different amounts of litter added for treated and untreated litter was intended to help standardize by the predicted dry mass (Barlocher 2005).

Thirty-two litterbags of treated and untreated litter were buried at a depth of 5 cm around the perimeter of untreated willow shrubs in the control area. Litterbags were buried in adjacent strips that alternating between litterbags with treated and untreated leaves. They were buried with at least 1 m between litterbags with leaves of the same treatment and 2 m between litterbags with leaves of different treatments.

Eight randomly selected litterbags from each treatment were recovered at 58 29, 90, 280 days after treatment (DAT), which occurred on August 2, 2017. Upon recovery, litterbags were stored individually in Ziploc bags and transported in a cooler with icepacks to the laboratory. The litter was brushed of soil particles, exogenous organic matter, and visible soil invertebrates (Lucisine et al. 2015). The litter from five litterbags from each treatment at all four time points were then weighed to the nearest 0.01 g for wet weight mass loss determination, oven dried at 60 °C, and reweighed to determine the dry mass (Yang et al. 2005). All mass values for determination of mass loss were corrected to dry weight. Mass loss comparison was determined based on the OECD

guidance document on the breakdown of organic matter in litterbags using the formula in Section 5.2.1 (Equation 5.1) and the following formula (Equation D.1) (OECD 2006):

Equation D.1

$$\% \text{ effect (mass loss)} = \frac{\text{mean mass loss control-mean mass loss treatment}}{\text{mean mass loss control}} \times 100$$

D.2. Statistical Analysis

Mass loss differences over four time points and between treatments were compared for litter buried in field litterbags 28 DAT (n = 5 per time point for each treatment). Time and treatment and the interaction between time and treatment were examined using a two-way Analysis of Variance (ANOVA) followed by TukeyHSD post hoc tests ($p<0.05$). Normality the model residuals were visually assessed.

D.3. Results

Litterbags were buried in a site between Debden and Big River, SK 28 days after treatment with a low-volume foliar application of Garlon XRT (triclopyr) on August 30, 2017. These litterbags were then recovered at 58 29, 90, 280 days following treatment, which occurred on August 2, 2017. Control leaves experienced consistency having higher mass loss for an overall effect of 41% (Figure D.1). There was no significant mass loss over time, nor was there an interaction between treatment and time.

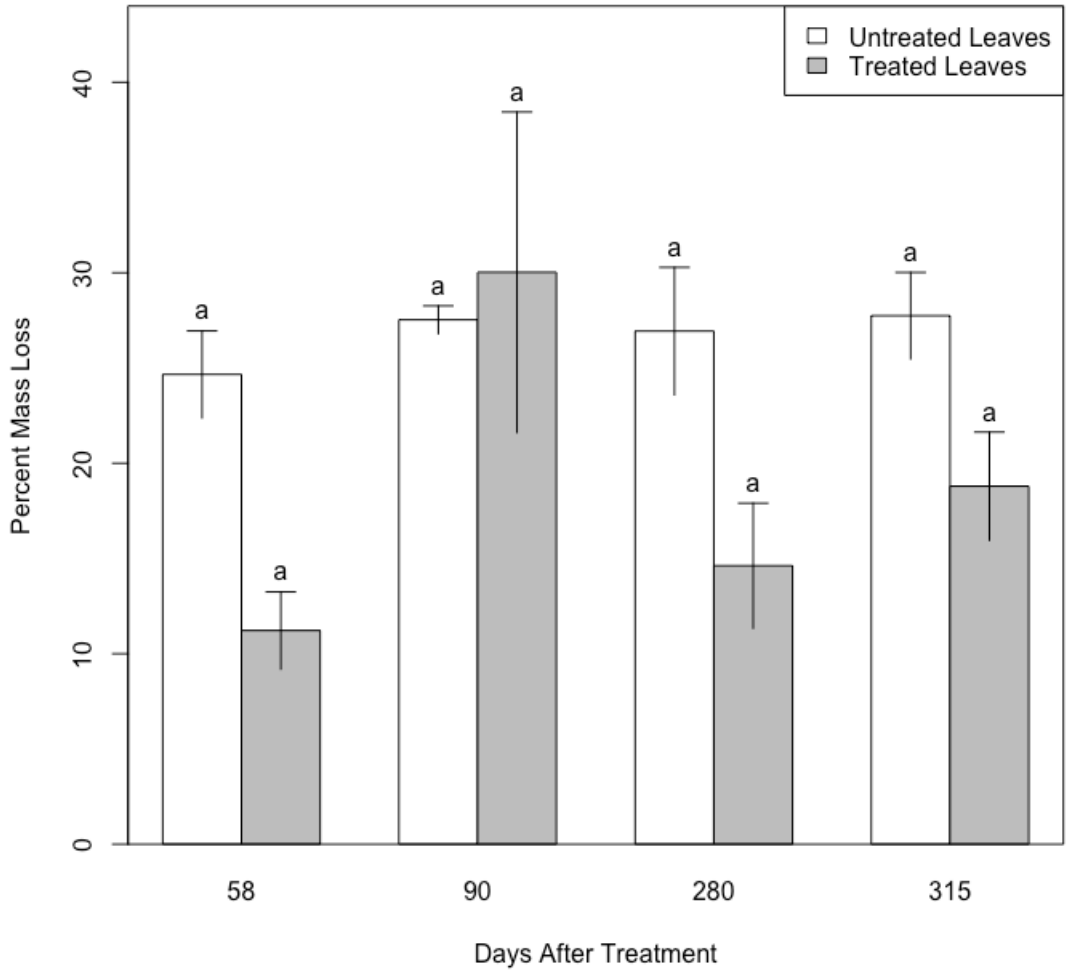


Figure D.1. Litter breakdown of willow leaves (*S. bebbiana*) buried in the field litterbags at 28 days after a low-volume foliar application of Garlon XRT (triclopyr). Mass loss expressed as a percentage (initial dry wt-final dry wt)/initial dry weight) and litter bags removed at four time points over a year. Different letters indicate a significant difference in mass loss over time (ANOVA, TukeyHSD<0.05).

D.4. DISCUSSION

At the time the litterbags were buried, control leaves were green and still contained much of their moisture whereas, treated leaves were brown and dried. Moisture content may have been a driving factor in breakdown of control leaves. Litter from litterbags recovered on the first time point were also rinsed before being dried, this can extract water soluble compounds further inflating mass loss values, especially in untreated litter.

References:

Barlocher F. 2005. Leaf Mass Loss Estimated by Litter Bag Technique. Pages 37-42 in Graca MAS, Barlocher F, and Gessner M, editors. Methods to Study Litter Decomposition: A Practical Guide. Springer, Netherlands.

Chomel M, Guittonny-Larchevque M, DesRochers A, Baldy V. 2016. Effect of mixing herbaceous litter with tree litters on decomposition and N release in boreal plantations. Plant and Soil **398**:229-241.

Lucisine P, et al. 2015. Litter chemistry prevails over litter consumers in mediating effects of past steel industry activities on leaf litter decomposition. Science of the Total Environment **537**:213-224.

OECD. 2006. Guidance Document on the Breakdown of Organic Matter in Litter Bags, Test and Assessment No. 56. Organization for Economic Co-operation and Development.

Yang L, Zhao YH, Zhang BX, Yang CH, Zhang X. 2005. Isolation and characterization of a chlorpyrifos and 3,5,6-trichloro-2-pyridinol degrading bacterium. Fems Microbiology Letters **251**:67-73.

APPENDIX E: NOMINAL AND MEASURED CONCENTRATIONS IN INVERTEBRATE AVOIDANCE TESTS

LIST OF TABLES

Table E.1. Nominal and measured concentrations for dose levels used to test the avoidance response of *Folsomia candida* and *Oppia nitens* to triclopyr. The nominal concentration represents the application dose of Garlon XRT (a.i. triclopyr) applied to leaves as gram active ingredient per area (hectare). Measured concentration in leaves represents micrograms active ingredient per gram of vegetation that was detected from herbicide analysis. Samples were analyzed in triplicate to assess consistency of dosing.

Table E.1. Nominal and measured concentrations for dose levels used to test the avoidance response of *Folsomia candida* and *Oppia nitens* to triclopyr. The nominal concentration represents the application dose of Garlon XRT (a.i. triclopyr) applied to leaves as gram active ingredient per area (hectare). Measured concentration in leaves represents micrograms active ingredient per gram of vegetation that was detected from herbicide analysis. Samples were analyzed in triplicate to assess consistency of dosing.

Invertebrate	Unique ID	Nominal Conc. (**g a.i. ha^{-1}**)	Measured Conc. (**ug a.i. g^{-1}**)
Collembola: *Folsomia candida*	CA: 0.25x-1	1133	172
	CA: 0.25x-2	1133	221
	CA: 0.25x-3	1133	258
	CA: 0.5x-1	2265	363
	CA: 0.5x-2	2265	345
	CA: 0.5x-3	2265	308
	CA: 1x-1	4530	980
	CA: 1x-2	4530	1036
	CA: 1x-3	4530	1091
	CA: 1.5x-1	6795	1210
	CA: 1.5x-2	6795	1230
	CA: 1.5x-3	6795	1450
	CA: 2x-1	9060	1900
	CA: 2x-2	9060	1740
	CA: 2x-3	9060	1785
	CA: 2.3x-1	10570	2225
	CA: 2.3x-2	10570	2300
	CA: 2.3x-3	10570	2375
	CA: 2.7x-1	12080	2750
	CA: 2.7x-2	12080	2892
	CA: 2.7x-3	12080	2625
	CA: 3x-1	13590	3121
	CA: 3x-2	13590	3050
	CA: 3x-3	13590	2944
	CA: 3.3x-1	15085	3767
	CA: 3.3x-2	15085	3750
	CA: 3.3x-3	15085	3664
	CA: 3.7x-1	16625	4038
	CA: 3.7x-2	16625	3861
	CA: 3.7x-3	16625	4000
Mites: *Oppia nitens*	MA: 0.5x-1	2265	425
	MA: 0.5x-2	2265	516
	MA: 0.5x-3	2265	475
	MA: 1x-1	4530	1170
	MA: 1x-2	4530	1078
	MA: 1x-3	4530	1110
	MA: 2.5x-1	11325	2679

MA: 2.5x-2	11325	2621
MA: 2.5x-3	11325	2446
MA: 3x-1	13590	3038
MA: 3x-2	13590	2931
MA: 3x-3	13590	3100
MA: 3.3x-1	15085	3656
MA: 3.3x-2	15085	3395
MA: 3.3x-3	15085	3575
MA: 3.7x-1	16625	4292
MA: 3.7x-2	16625	4194
MA: 3.7x-3	16625	4313
MA: 4.3x-1	19615	4563
MA: 4.3x-2	19615	4690
MA: 4.3x-3	19615	4604
MA: 4.7x-1	21155	5347
MA: 4.7x-2	21155	5194
MA: 4.7x-3	21155	4792

APPENDIX F: INFORMATIONAL POSTERS

Working in partnership with SaskPower and the Lac La Ronge Indian Band, this project was designed to address some of the herbicide toxicity concerns of local communities. Members of the Lac La Ronge Indian Band, SaskPower, and the Ministry of Environment participated in a final workshop facilitated by the University of Saskatchewan team. Informational posters in English and Cree were developed by Alix Conway and Katherine Stewart with my assistance and includes the results of the research in this project.

POSTER LIST

How can herbicides affect wildlife and the environment?

Herbicides and ecosystem health

How can herbicides affect wildlife and the environment?

Wildlife can be affected by herbicides if they are exposed once (acute toxicity) or if they are exposed multiple times (chronic toxicity). In general, triclopyr is considered to be of **low toxicity to mammals** and highly unlikely to cause death following a single exposure. For **fish, amphibians, and aquatic invertebrates** triclopyr can be **highly toxic** and cause death following a single exposure.

Garlon (triclopyr) is not expected to kill animals that eat plants that have been sprayed.

namōtha piskisīsak kita nipahīkwak Garlon kīspin micītwawi anihi kākisōpacikātikīk

Chronic exposure to herbicides can cause lower body weight of offspring and negative effects on animals' kidneys. However, **chronic exposure is not expected for wildlife browsing on rights-of-way plants.**

Triclopyr Break Down in Plants

Triclopyr breaks down relatively rapidly in plants with nearly all the herbicide gone within 30-45 days following spraying.

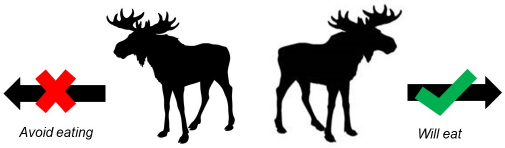
Foraging Wildlife

Plants treated with triclopyr by spraying or basal bark application are wilted within ~7 days and are dried and shriveled by ~30 days. **Wildlife are unlikely to eat these damaged plants** since they can forage in areas off the sprayed right-of-way.

On-going Exposure

Herbicide is not sprayed for multiple years in a row which **means wildlife are highly unlikely to experience multiple exposure events.**

Dried/dead willow leaves 30 days after herbicide spray.



Undamaged, healthy willow off of ROW or on untreated ROW.

Garlon RTU is **currently not used within 30m of water.** Contamination of surface waters can result from spray drift, runoff, or leaching.

namōtha Garlon isowīpahikātīw kisiwāk nipihk

It is important that Garlon RTU does not contaminate aquatic systems because even small amounts of herbicide can have harmful effects.

If **the necessary precautions to avoid herbicide entering aquatic systems are taken**, the risk to aquatic environments from application of triclopyr on rights-of-way would be minimal.

Our research shows that **triclopyr does not travel far when applied using a low-pressure backpack sprayer**. We detected herbicide in only half of the samples 2m from the site of foliar application. Where we did detect herbicide, **concentrations were very low** and are unlikely to have negative effects in the environment. **Basal bark application helps reduce the chance of herbicide entering the soil or water**

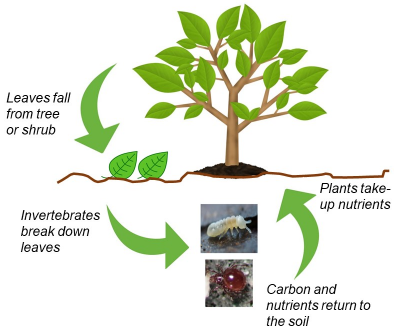
īka askik īkwa nipihk kita ispathik Garlon, īyakoci kicowāk ana mistihk kāsopatit

Questions? Contact: Dr. Katherine Stewart
Phone: (306) 966-1916; Email: Katherine.stewart@usask.ca

Herbicides and ecosystem health

Triclopyr has low toxicity in soils and to wildlife and humans, but it may still affect the overall health of the environment. However, our research to date does **not show an impact of Garlon on ecosystem health when applied by low volume backpack spraying or the basal bark method.**

When leaves fall from shrubs and trees they land on the surface of the soil, where they break down over time. This is a very important process that returns energy and nutrients to the soil, keeping the ecosystem healthy.



Soil invertebrates, which are tiny organisms that live in the soil, **help to break down leaves and release nutrients.** These organisms are useful for testing toxicity because they are found in all boreal forest soils.

Two different kinds of soil invertebrates. Left: Springtail, Right: Mite

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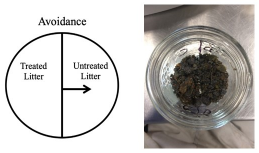
Herbicides could impact ecosystem health if: i) they are toxic to soil invertebrates, ii) if soil invertebrates avoid leaves sprayed with herbicides or iii) if the break down of sprayed leaves is slower or faster than normal.

i) Previous work found that **Garlon (triclopyr) does not have a direct negative effect on soil invertebrates**. The invertebrates were only killed when herbicide was applied at a rate higher than the maximum concentration allowed.

īkā apoh īmīskwāpiminākōsicik awīyakisak askik kā ayācik kāsikwatahkwaw nīpīyā, namōtha nāntow itskākwak Garlon

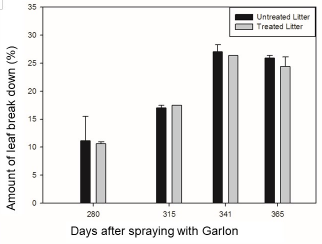
ii) We sprayed leaves with Garlon (triclopyr) and watched to see if the invertebrates avoided the leaves. **At the maximum concentrations allowed for Garlon, soil invertebrates did not avoid the leaves.**

namōtha ātāwithītāmwak anihi nīpīya kaki sōpācikatīkik



Invertebrates will move to untreated (no herbicide) leaves if they do not like the herbicide.

iii) We took leaves that had been sprayed with Garlon (triclopyr) and leaves that had not been sprayed with herbicide, weighed them and buried them in mesh bags.



After 10 months to 1 year later we dug up the bags and weighed them again. We determined how much of the leaves had broken down. **There was no difference in leaf breakdown between the sprayed and unsprayed leaves.**

Questions? Contact: Dr. Katherine Stewart
Phone: (306) 966-1916; Email: Katherine.stewart@usask.ca